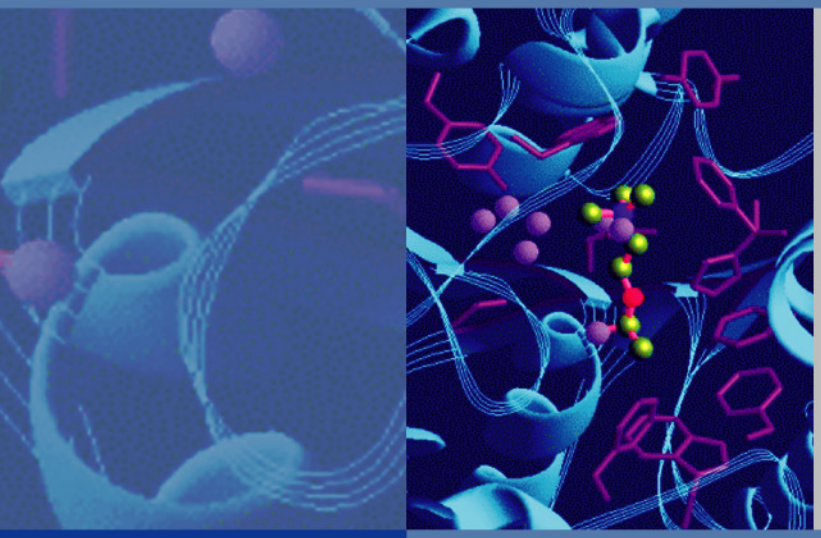


Xcalibur

LCquan

Quantitative Analysis

User Guide

XCALI-97166 Revision D August 2007

Thermo SCIENTIFIC

© 2007 Thermo Fisher Scientific Inc. All rights reserved.

Microsoft®, Access®, Excel®, and Notepad® are registered trademarks of Microsoft Corporation. Adobe® and Acrobat® are registered trademarks of Adobe Systems Incorporated. Oracle® is a registered trademark of Oracle Corporation. Citrix Presentation Server™ is a trademark and Citrix® is a registered trademark of Citrix Systems Incorporated.

All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Thermo Fisher Scientific Inc. provides this document to its customers with a product purchase to use in the product operation. This document is copyright protected and any reproduction of the whole or any part of this document is strictly prohibited, except with the written authorization of Thermo Fisher Scientific Inc.

The contents of this document are subject to change without notice. All technical information in this document is for reference purposes only. System configurations and specifications in this document supersede all previous information received by the purchaser.

Thermo Fisher Scientific Inc. makes no representations that this document is complete, accurate or error-free and assumes no responsibility and will not be liable for any errors, omissions, damage or loss that might result from any use of this document, even if the information in the document is followed properly.

This document is not part of any sales contract between Thermo Fisher Scientific Inc. and a purchaser. This document shall in no way govern or modify any Terms and Conditions of Sale, which Terms and Conditions of Sale shall govern all conflicting information between the two documents.

Release history: Revision A - December 2006. Revision B - February 2007. Revision C - April 2007. Revision D - August 2007.

Minimum software requirements: LCquan 2.5.6; Xcalibur 2.0.6; TSQ Quantum 1.4; LC Devices 2.0.2; Thermo PAL 1.0; Microsoft Windows XP Professional SP 2; Microsoft Office 2003

For Research Use Only. Not regulated for medical or veterinary diagnostic use by U.S. Federal Drug Administration or other competent authorities.

Contents

Preface . . . . . **vii**
About This Guide vii
Related Documentation vii
Special Notices viii
Contacting Us viii

Chapter 1 **Introduction** **1**
About Quantitative Analysis 2
Considering the Variables of Quantitative Analysis by LC/MS/MS 3
Using LC for Analyte Separation 3
Using MS/MS for Analyte Detection 4
Quantitative Analysis Techniques 5
Using External Standards for Quantitative Analysis 7
Using Internal Standards for Quantitative Analysis 8
Sample Types 9
Standards 9
Unknowns 9
QCs 10
Blanks 10
Introduction to Quantitative Analysis with LCquan 10
Overview of LCquan 10
LCquan Folder Structure 16
Acquiring and Processing Data with LCquan 17
Using This Manual 18
LCquan for Citrix 19

Chapter 2 **Preparing to Perform Quantitative Analysis of Steroids Data** **21**
Creating a New Study and Workbook 22
Reviewing the Instrument Setup View 25
Creating Instrument Methods 26
Importing an Existing Instrument Method 27

Reviewing the Acquisition View 27
Setup Sequence Page 28
Naming Components and Assigning Calibration Levels 36
Run Sequence Dialog Box 43
Acquisition View – Status Page 45
Processing Data While Continuing to Acquire Data 50

Chapter 3 Exploring Your Data 51
About the Explore View 52
Opening a Raw File and Displaying the Chromatogram 52
Generating a Peak List 54
Identifying Components 57
Reviewing the Explore Results 59
Creating a Quan Component from the Peak List 61
Additional Features of the Review and Explore Results Page 62
Sample Selector 63
Locks 65
General Parameters Plot 66
Status Plot 67
Status Report 68
Review Displays 69
Exporting the Active Raw File to Qual Browser 70

Chapter 4 Creating a Processing Method 71
Opening the Create Method Page and Using the New Method Wizard 72
Specifying the Component Identification and Integration Parameters 75
Identifying the Internal Standard 75
Specifying Calibration Curve Parameters for the Target Compounds 75
Entering the Component Identification Parameters and Integrating the Peaks 77
Ion Ratio Confirmation of Analytes 83
Importing the Components and Levels into the Acquisition Sequence 84
Saving the Processing Method 85
Importing an Existing Processing Method 85

Chapter 5 Creating a Processing Sequence 87
Opening the Create Sequence Page and Using the New Processing Sequence Wizard 88
Associating Raw Files with Sample Types 89
Reviewing the Processing Sequence 94
Further Information: Additional Ways to Define the Processing Sequence 95
Importing an Existing Sequence 95
Copying the Acquisition Sequence 96
Further Information: Editing the Processing Sequence 97

Chapter 6 Processing the Raw Files and Reviewing the Analytical Results 99
Processing the Raw Files 100
Reviewing the Calibration Curve and QCs 100
Opening the Survey Page 100
Excluding a Calibration Standard from the Calibration Curve 107
Modifying the Peak Detection and Integration Settings 109
Integrating Peaks Manually 110
Reviewing All Results 112
Opening the Review All Results Page 112
Reviewing the Unknowns 114
Creating and Reviewing Reports 116
Locking the Workbook 121
Saving the Workbook and Exiting LCquan 121

Appendix A LCquan Flow Diagrams 123

Preface

About This Guide

LCquan™ is part of the Xcalibur® mass spectrometry data system. This user guide describes how to use LCquan to perform quantitative analysis of compounds.

Related Documentation

The following LCquan manuals are available on the LCquan software CD as PDF files:

- *LCquan Administrator Guide* describes how to configure the software for compliance with 21 CFR Part 11.
- *LCquan User Guide* describes how to use LCquan to perform quantitative analysis of compounds.

❖ To view the LCquan manuals

Go to **Start > Programs > Xcalibur > Manuals > LCquan**.

The Help contains an LCquan Tutorial that provides an overview of how to use LCquan with an example data set.

❖ To open the LCquan Help

From the LCquan window, choose **Help > LCquan Help**. To locate a particular topic, use the Help Contents, Index, or Search panes.

Special Notices

Special notices include the following:

IMPORTANT Highlights information necessary to avoid damage to software, loss of data, invalid test results, or information critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Helpful information that can make a task easier.

Contacting Us

There are several ways to contact Thermo Fisher Scientific.

❖ **To contact Technical Support**

Phone	800-685-9535
Fax	561-688-8736
E-mail	TechSupport.C+MS@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at www.mssupport.thermo.com.

❖ **To contact Customer Service for ordering information**

Phone	800-532-4752
Fax	561-688-8731
Web site	www.thermo.com/finnigan

❖ **To suggest changes to documentation or to Help**

- Fill out a reader survey online at www.thermo.com/lcms-techpubs.
- Send an e-mail message to the Technical Publications Editor at techpubs.finnigan-lcms@thermofisher.com.

Introduction

This chapter describes some of the basic principles and terminology of quantitative analysis[1] and provides a brief overview of quantitative analysis with LCquan. This chapter contains the following sections:

Contents

- About Quantitative Analysis
- Considering the Variables of Quantitative Analysis by LC/MS/MS
- Quantitative Analysis Techniques
- Sample Types
- Introduction to Quantitative Analysis with LCquan
- LCquan for Citrix

[1]For further information on the principles of quantitative analysis, refer to the following: *Mass Spectrometry: Principles and Applications*; de Hoffman, E., Charette, J., Stroobant, V.; Wiley: New York, 1996 and *Introduction to Mass Spectrometry*, 3rd ed.; Watson, J.T., Lippincott-Raven: Philadelphia, PA, 1997.

About Quantitative Analysis

Quantitative analysis is the process of measuring the amount of a particular component in a sample.

In some applications, such as a clinical trial, you might be seeking the maximum possible accuracy from your measurements. Time and cost of analysis are less important than achieving the highest possible standards in precision and accuracy. This form of measurement is called ***quantitative analysis***.

In other applications, such as in trace analysis, you might only want to estimate the quantity of a component. It might be sufficient to know that the component is present at a level either significantly higher or significantly lower than a defined threshold. For example, it is generally not important to know whether a patient has overdosed 15 or 20 times above a prescribed limit, but simply that the limit has been exceeded. In such cases, a rapid measurement is required rather than a precise one. This form of measurement is generally called ***semi-quantitative analysis***.

This chapter discusses quantitative analysis in detail. Quantitative analysis consists of the following steps:

- Preparing samples
- Developing a suitable chromatographic method
- Calibrating the mass spectrometer's response
- Analyzing the samples
- Reviewing the results

It is beyond the scope of this manual to describe sample preparation and chromatographic method development. This manual assumes that you have achieved these important prerequisites to high quality quantitative analysis. Refer to the *Getting Started* and *Hardware* manuals for your autosampler, LC pump or MS pump, and mass spectrometer for guidance in these areas.

Considering the Variables of Quantitative Analysis by LC/MS/MS

The combination of liquid chromatography (LC) and mass spectroscopy (MS) sets up a unique set of considerations. Chemists who are familiar with quantitative analysis using one or the other of these analytical tools can find the information in the following topics useful:

- Using LC for Analyte Separation
- Using MS/MS for Analyte Detection

Using LC for Analyte Separation

Chemists who work with LC are accustomed to developing methods for their analytes of interest that take into account a variety of parameters. The following LC parameters might be considered for optimum separation of compounds:

- Composition of stationary phase
- Column diameter
- Column length
- Column usability over time
- Solvent(s) or composition of mobile phase
- Flow rates
- Isocratic or gradient (ramped) solvent compositions

Often the only measurable result of optical detectors that LC chemists can use to identify an analyte is the retention time at a specified wavelength. Therefore to quantitate compounds accurately, the compound peaks must be distinctly separated from each other in time. By carefully optimizing the LC variables listed above, compounds can be successfully separated and detected with an optical detector such as an ultraviolet (UV) or photo diode array (PDA) detector.

In contrast to optical detectors, mass spectrometers can often successfully detect individual analytes even when two or more compounds coelute as a single chromatographic peak. Coeluting compounds are ionized in the mass spectrometer, and the parent or fragment ions specific to the analyte(s) are detected and measured for quantitative analysis. Because the mass spectrometer detection is so specific, the chromatographic resolution can be reduced and the run times can be shortened.

Using MS/MS for Analyte Detection

Chemists who work with a mass spectrometer interfaced to an LC should consider the following variables to optimize detection of ions by MS/MS:

- Solution chemistry and polarity of the analyte of interest
- Probe selection (H-ESI, ESI, APPI, or APCI)
- Collision energy to fragment parent ions inside the mass spectrometer (optimized by experiment)

The LC provides a stream of analyte solution (eluate) into the mass spectrometer, where the analyte is detected. Consideration must be given to the content of modifiers, salts, and contaminants in the eluate. Specifically, you must ensure that what goes into the inlet of the mass spectrometer does not suppress ionization of the compound of interest. Salt concentrations above 10 mM and strong acids and bases will damage the LC column. Modifier concentrations greater than 10 mM are not usually necessary for chromatographic stability and can suppress ionization of other compounds. Whenever possible, it is best to use volatile modifiers.

Volatile modifiers include the following:

- Acetic acid
- Ammonium acetate
- Ammonium formate
- Ammonium hydroxide
- Formic acid
- Trifluoroacetic acid

Your analyte solution can contain neutral particles or ions. If the solution contains ions, the ions can carry a single charge or multiple charges. The number of charges depends on the structure of the analyte and the composition and pH of the mobile phase. Solvent systems are generally composed of organic solvents, water, and volatile modifiers. The ionization characteristics of your analyte should influence your decisions about the optimum pH of your solvent system and about which probe to use at the interface of the LC and the mass spectrometer.

With the TSQ Quantum series, LTQ series, or LCQ series instruments, you can use either the H-ESI probe, the ESI probe, the APCI probe, or the APCI/APPI probe at the interface of the LC. The choice of probe depends on the class of compound that you want to analyze, and to a small extent, on the flow rate of your experiment. In general, the APCI and the APCI/APPI probes accommodate higher flow rates and molecules of a less polar nature than does the ESI probe. Refer to the “Introduction” chapter in the *Getting Started* manual for your mass spectrometer for a discussion of factors that can influence your decision about flow rates and about which probe to use for optimum transfer of ions into the mass spectrometer.

After the ions are inside the mass spectrometer and the parent ions are isolated, collision energy is applied to dissociate the parent ions into product ions (MS/MS). The relative collision energy for a particular analysis depends on the structure of the compound that you are analyzing. For this reason, you optimize the collision energy on your analyte of interest in a tuning experiment. Refer to the *Getting Started* manual for your mass spectrometer for the tuning procedure that describes how to optimize the collision energy.

Quantitative Analysis Techniques

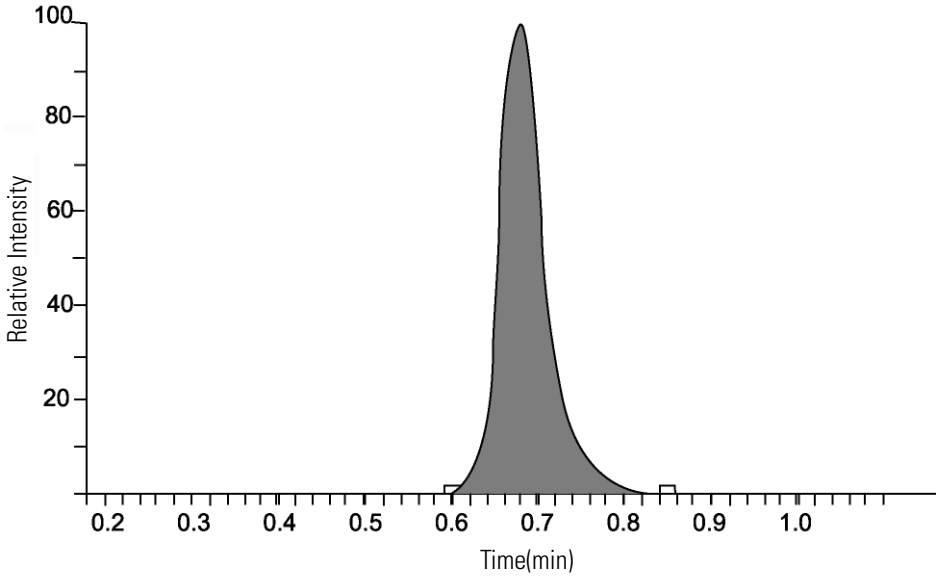
To carry out quantitative analysis, you need to evaluate the instrument's response to known amounts of the target component. Response is based on either the height of the chromatogram peak, or more commonly, the area under the peak's profile. See Figure 1. In both cases the baseline of the detected peak must be taken into account.

Instrument response is generally measured with several samples commonly called ***standards***, or calibration standards. These standards must cover a suitably wide range of concentrations or amounts and must bracket the range of expected concentrations in the unknown. Responses to these standards are plotted in a graph called a ***calibration curve***. Ideally, this curve should correspond to the equation of a straight line to ensure the highest degree of precision.[2]

Fitting an equation to the calibration curve with a user chosen method (for example, a least squares regression) provides a ***response factor*** - a comparative measure of the response of the mass spectrometer to a component. It is based on the amount of sample injected and the resulting peak area or peak height. Thus, the response factor gives an intuitive and quantitative measure of how responsive or sensitive the mass spectrometer is to a certain component.

[2] *Mass Spectrometry: Principles and Applications*; de Hoffman, E., Charette, J., Stroobant, V.; Wiley: New York, 1996; p 162.

Figure 1. Integrated chromatographic peak



Quantitative Analysis of samples containing unknown amounts of the target component is achieved by first calculating the peak area or height and then computing and applying the appropriate response to the equation derived from the calibration curve. This provides an estimate of the amount of the unknown component. The precision of the measurement depends on the quality and, to a lesser extent, the quantity of the calibration data.

The ***detection limit*** of the quantitative analysis method is the lowest concentration of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The ***lower and upper quantification limits*** are the lowest and highest concentrations of analytes in a sample that can be measured with an acceptable level of accuracy and precision.
In an analytical method, the highest concentration calibration standard defines the upper quantification limit. The ***quantification range*** is the range of concentration between the lower and upper quantification limits (including these limits) that can be reliably and reproducibility quantified with acceptable levels of accuracy and precision through the use of a concentration-response relationship.

There are two basic quantitative analysis techniques:

- External standard quantitative analysis
- Internal standard (ISTD) quantitative analysis

The chosen method determines the manner in which response is calculated, both for the generation of the calibration curves and for subsequent quantitative analysis. These methods are described in the following topics:

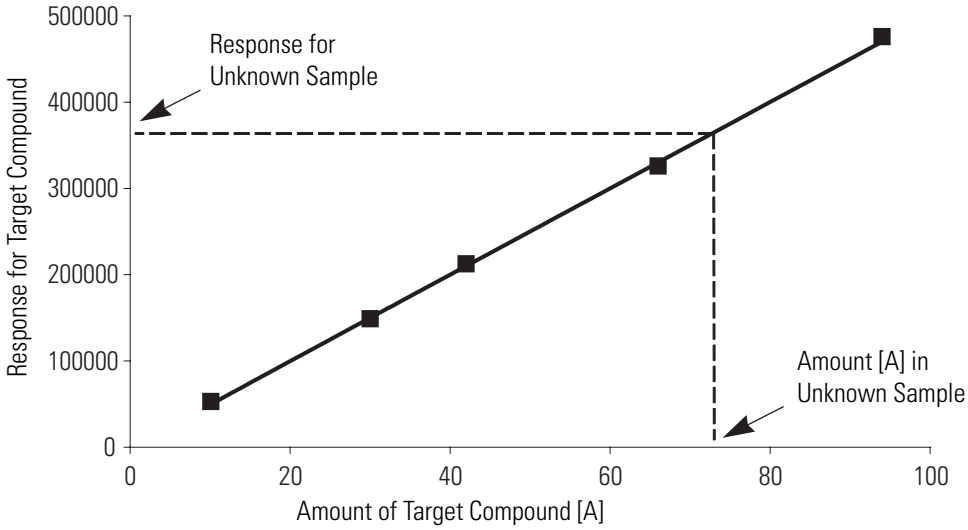
- Using External Standards for Quantitative Analysis
- Using Internal Standards for Quantitative Analysis

Using External Standards for Quantitative Analysis

An ***external standard*** is a separate sample that contains the compound of interest at a known concentration in solution. In the external standard quantitative analysis technique, a series of standards are analyzed and a calibration curve is constructed by plotting the magnitude of the detector response as a function of the external standard concentration. The unknown sample is then analyzed, and the concentration is determined by matching the magnitude of the detector response with that on the calibration curve. See Figure 2.

Using external standards is advantageous if various components in a sample are being analyzed, and if all compounds of interest can be assayed by using a single set of external standards. This approach offers time- and cost-effective quantitative analysis, although it is not capable of achieving the very highest precision and accuracy. Variations in analyte and solution stability, injection reproducibility, and matrix interference lead to lower precision levels in the external standard method than in the internal standard method.

Figure 2. Calibration curve generated by using an external standard



Using Internal Standards for Quantitative Analysis

An ***internal standard (ISTD)*** is a component that is added to a sample to act as a response reference for one or more non-ISTD components in the sample. The concentration or amount of an ISTD in any standard or unknown sample remains constant.

Because quantitative mass spectrometric analysis usually involves multiple steps, the total error in the analysis results from the accumulation of the errors at each step. In general, sample handling errors account for a larger fraction of the total error than do mass spectrometer errors. Fortunately, the internal standard method can reduce both sources of error. For example, internal standards can correct for variations in a component's peak area that are caused by the following:

- Injection irreproducibility
- Changes in analyte solution volume
- Matrix and coeluter interference (both suppression and enhancement)
- System instability
- Variations in the source conditions

For maximum precision, the ISTD component should be added as early as possible to the start of the sample workup, particularly in those quantitative methods that require sample manipulations such as extraction, cleanup, and dilution. Since the ISTD and non-ISTD components are analyzed together, the internal standard quantitative analysis approach has the advantage that it corrects for injection and other sample handling errors. The ISTD must behave chemically in an identical or similar manner to the target compound through the extraction, cleanup, and analytical processes.

The ISTD component can also be added as the last step of sample preparation prior to the sample's use to compensate for fluctuations in the reproducibility of the sample injection.

In general, ISTDs are used in a quantitative analysis experiment as follows:

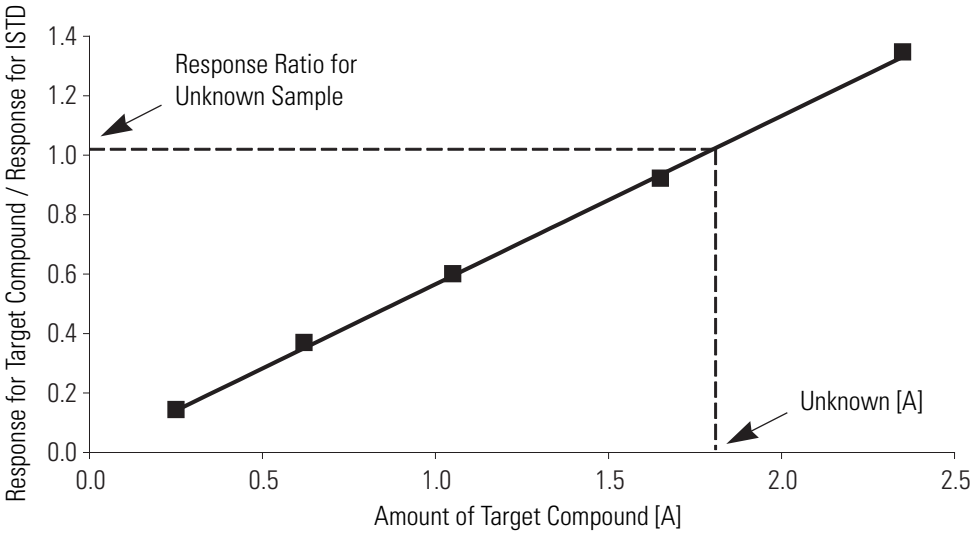
1. A series of standard solutions containing known concentrations of the target compound and ISTD are analyzed. Then, the ratio of the target compound and the ISTD detector responses are plotted as a function of the corresponding ratio for the two quantities present in the solution.
2. A fixed amount of the ISTD is added to each sample prior to any manipulation. Then, after the samples are prepared and analyzed, the quantity of the target compound present in an unknown sample can be obtained from the calibration curve. See Figure 3.

Ideally, an ISTD should be closely related to the target component in terms of its physical and chemical properties. It must be pure, not present in the sample, and inert towards the components of the sample. ISTD components are typically analogs, homologues or isomers of the target non-ISTD component. An ideal ISTD is a structural or isotopically-labeled analog of one of the target components. Stable isotope-labeled ISTDs act almost identically to the

analyte throughout sample manipulation and with regard to ionization tendencies and fragmentation. Internal standards labeled with two or more deuterium (D) atoms are frequently used for LC/MS.

There can be any number of ISTD components in a sample but each non-ISTD component can be calibrated against only one ISTD.

Figure 3. Calibration curve generated by using an internal standard



Sample Types

Each quantitative analysis consists of a number, or ***sequence***, of samples. The sequence represents the order of sample analysis. A quantification sequence contains:

- One or more ***standards***
- One or more ***unknown*** samples

For more demanding applications, you can also use optional ***quality control (QC)*** samples and ***blank*** samples.

Standards

A calibration standard is a sample containing known amounts of all target components. The purpose of a standard is to measure the response of the instrument to the target components so that a calibration curve can be generated for each component.

Unknowns

An unknown sample is one containing unknown amounts of the target components. LCquan performs quantitative analysis on any sample defined as an unknown sample.

QCs

A QC sample contains a known amount of one or more specific target compounds. QC samples are placed in the sequence so that quantitative analysis results can be tested for quality assurance purposes. After the QC sample is analyzed, the measured quantity is compared with the expected value and an acceptability range. The quantitative analysis of a QC sample is classified as *passed* if the difference between the observed and expected quantities is within the user defined tolerance. A QC sample is classified as *failed* if the difference between the observed and expected quantities is outside the defined tolerance.

Blanks

A blank sample contains no target components but might contain an ISTD when the internal standard quantitative analysis technique is being used. The analysis of a blank sample allows you to confirm that there are no residual components in the solvent system that can cause erroneous results.

Introduction to Quantitative Analysis with LCquan

LCquan is a complete quantitative analysis software package that enables you to acquire data specifically for an analyte of interest, to quantitate the results, and to produce reports.

This section contains the following topics:

- Overview of LCquan
- LCquan Folder Structure
- Acquiring and Processing Data with LCquan
- Using This Manual

Overview of LCquan

LCquan is divided into four main views: Instrument Setup, Acquisition, Explore, and Quantitate. By using these views, you can develop Instrument Methods, acquire data, explore data, and process data files.

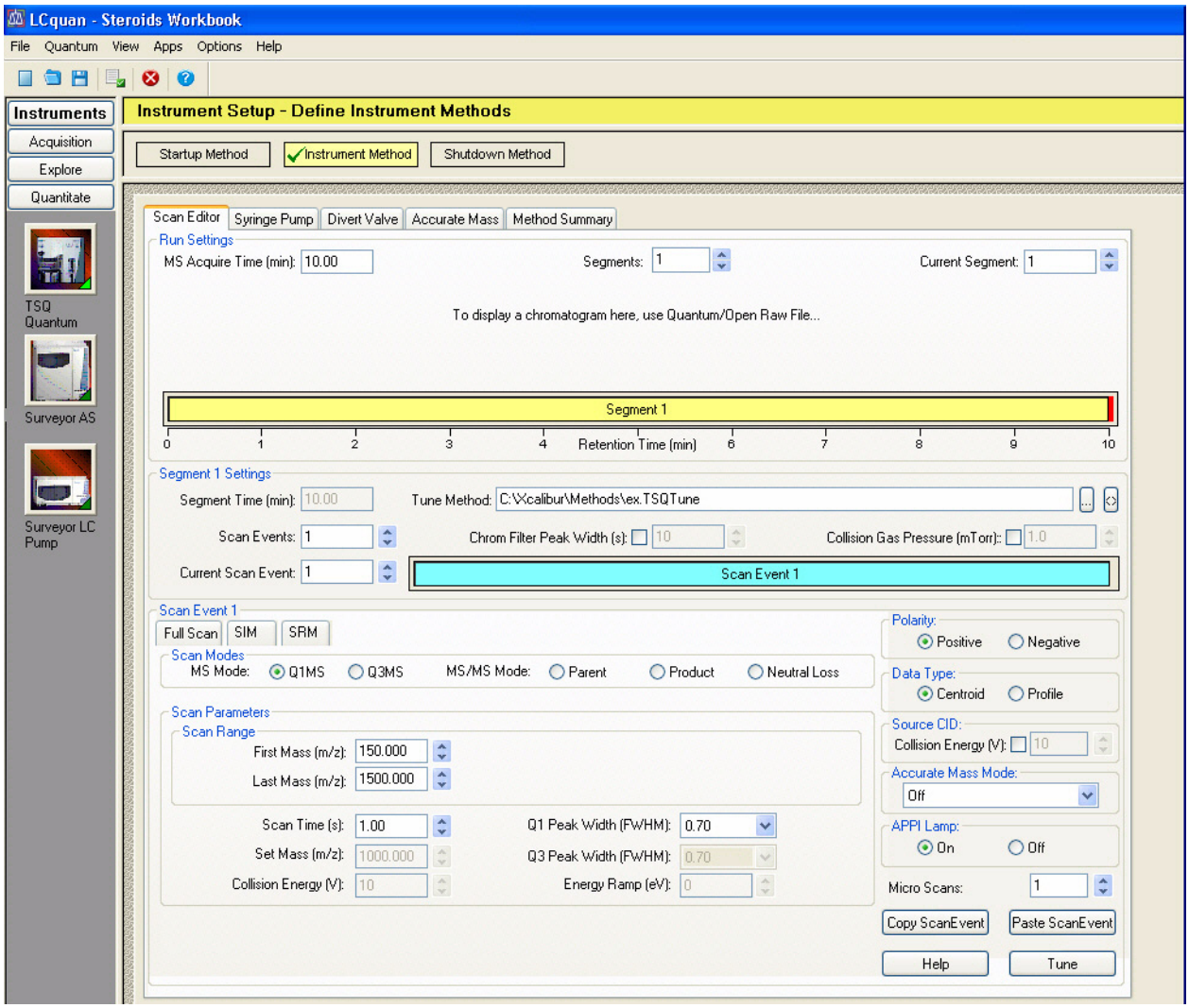
Instrument Setup View

The Instrument Setup view of LCquan (see Figure 4) enables you to develop Instrument Methods for the instruments that you have selected by using the Xcalibur Instrument Configuration window. An Instrument Method is a set of experimental parameters and operating settings for a specific instrument, such as an autosampler, LC pump or MS pump, mass spectrometer, divert valve, or syringe pump. Within the Instrument Setup view you can create new methods or modify existing methods. You can also import previously collected data files to help you set up time segments and scan events based upon the data acquired. In general, the Instrument Setup view within LCquan functions like the Instrument Setup window of the Xcalibur Home Page.

The Instrument Setup view contains separate pages for each instrument that you have selected by using the Xcalibur Instrument Configuration window.

Note The Instrument Setup view is not available in LCquan for Citrix.

Figure 4. Instrument Setup view



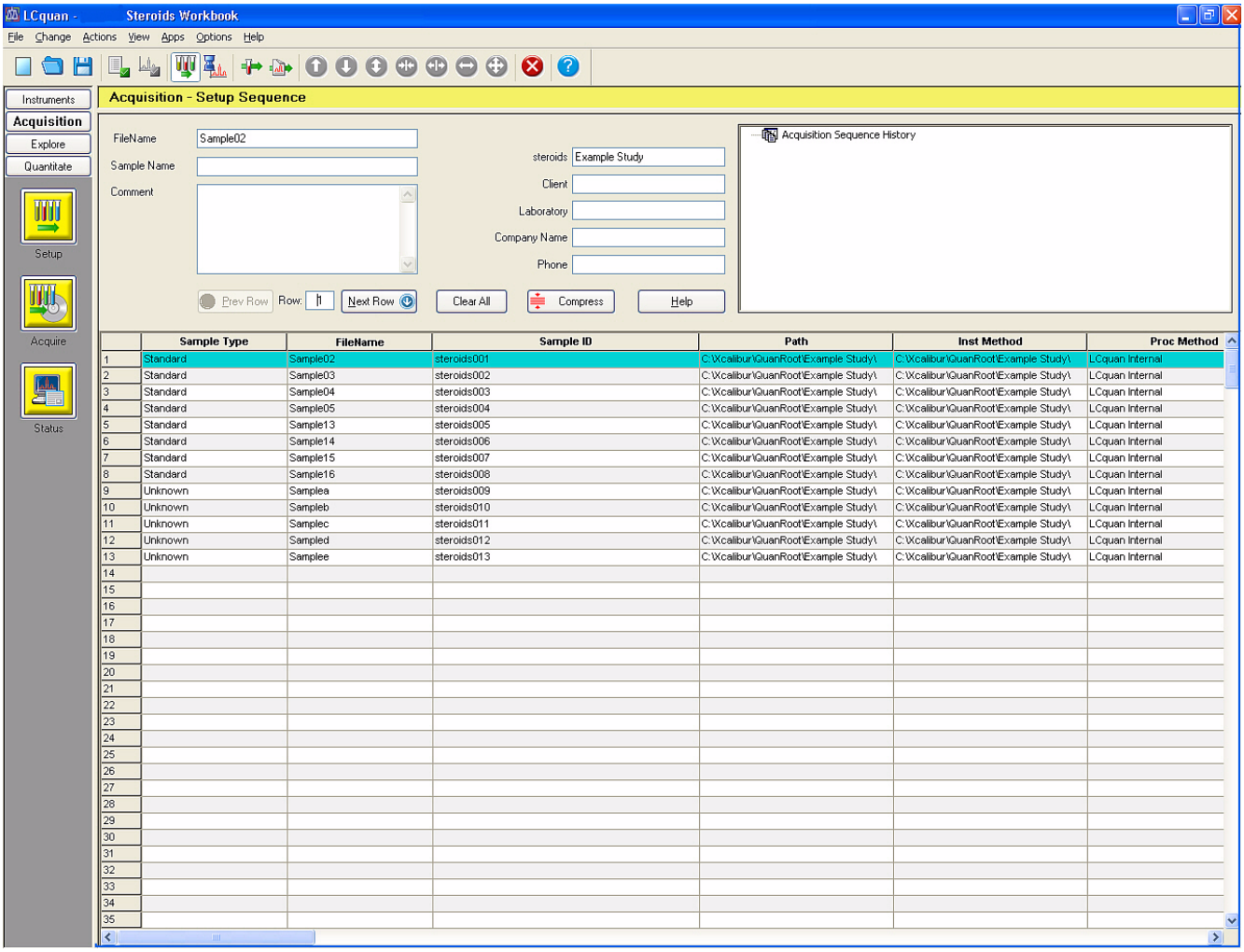
Acquisition View

The Acquisition view of LCquan (see Figure 5) allows you to set up a sequence of samples for acquisition. The sequence can contain unknown samples, calibration standard samples, quality control samples, and blank samples. Once you have defined a sequence, you can specify acquisition options and processing action options for the sequence and then start running it. The Acquisition view also contains a real-time display so that you can watch updates of the acquisition status and the data being acquired.

The Acquisition view contains the Sequence Setup page, the Run Sequence dialog box, and the Status page.

Note The Acquisition view is not available in LCquan for Citrix.

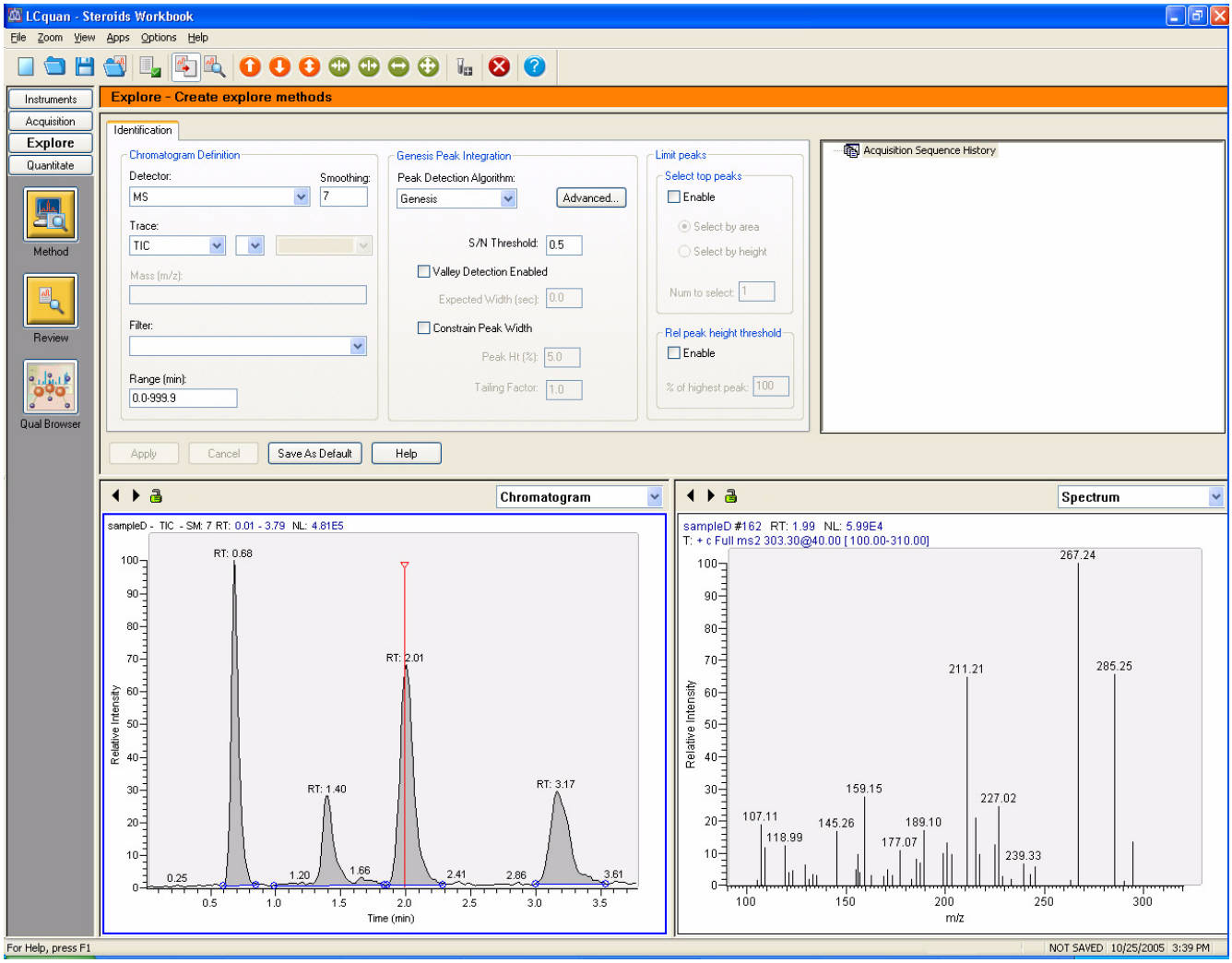
Figure 5. Acquisition view – Setup Sequence page


LCquan - Steroids Workbook
File Change Actions View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Setup
Acquire
Status
Acquisition - Setup Sequence
FileName Sample02
Sample Name
Comment
steroids Example Study
Client
Laboratory
Company Name
Phone
Acquisition Sequence History
Prev Row Row: 1 Next Row
Clear All
Compress
Help
Sample Type FileName Sample ID Path Inst Method Proc Method
1 Standard Sample02 steroids001 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
2 Standard Sample03 steroids002 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
3 Standard Sample04 steroids003 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
4 Standard Sample05 steroids004 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
5 Standard Sample13 steroids005 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
6 Standard Sample14 steroids006 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
7 Standard Sample15 steroids007 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
8 Standard Sample16 steroids008 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
9 Unknown Samplea steroids009 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
10 Unknown Sampleb steroids010 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
11 Unknown Samplec steroids011 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
12 Unknown Sampled steroids012 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal
13 Unknown Samplee steroids013 C:\Xcalibur\QuanRoot\Example Study\ C:\Xcalibur\QuanRoot\Example Study\ LCquan Internal

Explore View

The Explore view allows you to display a multi-peak chromatogram, for a single sample, and then to experiment with peak detection and integration parameters to see how they affect the chromatogram. In the Explore view, you determine the optimum parameters to be used in the Processing Method. LCquan can also generate a peak list, from which you can generate quan components to be used in the Processing Method. The Create Explore Method page of the Explore view is shown in Figure 6.

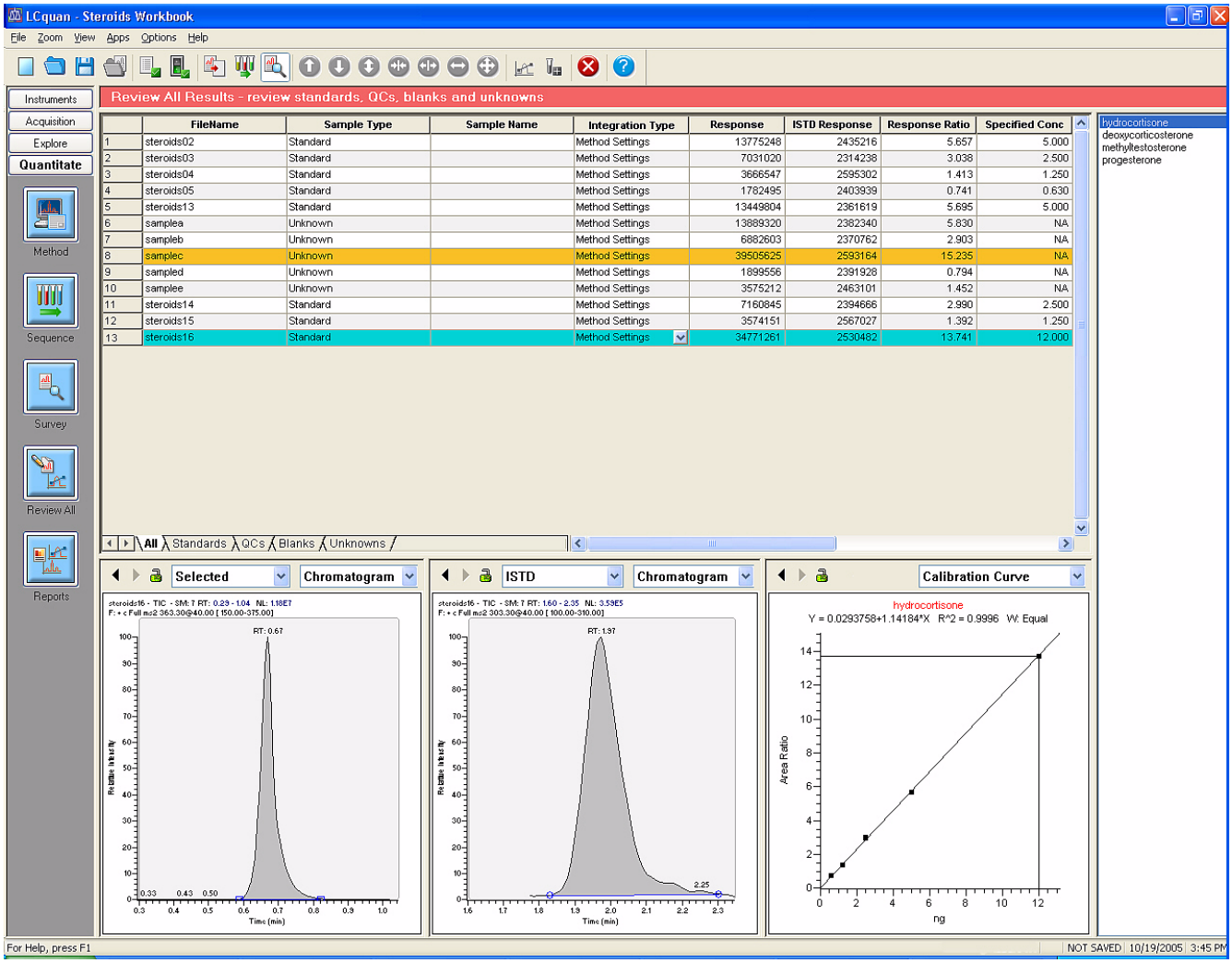
Figure 6. Explore view – Create Explore Method page, showing a multipeak chromatogram



Quantitate View

In the Quantitate view (see Figure 7), LCquan steps you through a procedure for batch processing raw data files during quantitative analysis. The LCquan window incorporates an integrated calibration curve, peak integration, and results view that allows you to review and rework standards, QCs, and unknowns interactively in one window. You can also enter the Quantitate view while you are acquiring data to process the data acquired to date.

Figure 7. Quantitate view – Review All Results page


LCquan - Steroids Workbook
File Zoom View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Method
Sequence
Survey
Review All
Reports
Review All Results - review standards, QCs, blanks and unknowns
FileName | Sample Type | Sample Name | Integration Type | Response | ISTD Response | Response Ratio | Specified Conc
1 steroids02 Standard Method Settings 13775248 2435216 5.657 5.000
2 steroids03 Standard Method Settings 7031020 2314238 3.038 2.500
3 steroids04 Standard Method Settings 3666547 2595302 1.413 1.250
4 steroids05 Standard Method Settings 1782495 2403939 0.741 0.630
5 steroids13 Standard Method Settings 13449804 2361619 5.695 5.000
6 samplea Unknown Method Settings 13889320 2382340 5.830 NA
7 sampleb Unknown Method Settings 6882603 2370762 2.903 NA
8 samplec Unknown Method Settings 39505625 2593164 15.235 NA
9 sampled Unknown Method Settings 1899556 2391928 0.794 NA
10 samplee Unknown Method Settings 3575212 2463101 1.452 NA
11 steroids14 Standard Method Settings 7160845 2394666 2.990 2.500
12 steroids15 Standard Method Settings 3574151 2567027 1.392 1.250
13 steroids16 Standard Method Settings 34771261 2530482 13.741 12.000
hydrocortisone
deoxycorticosterone
methyltestosterone
progesterone
All Standards QCs Blanks Unknowns
Selected Chromatogram
RT: 0.67
ISTD Chromatogram
RT: 1.97
Calibration Curve
hydrocortisone
Y = 0.0293758+1.14184*X R^2 = 0.9996 W: Equal
Area Ratio
ng
Time (min)
For Help, press F1
NOT SAVED 10/19/2005 3:45 PM

LCquan uses a Processing Method to automatically determine the concentration (or amount) of the sample being analyzed. The Processing Method measures the response of the detection system to the compounds in your sample by integrating the area under each peak.

After several known standards are measured, LCquan constructs a calibration curve, which it uses to accomplish the quantitative analysis of unknown samples. LCquan provides a number of options for fitting the calibration data to generate the calibration curve. You can have LCquan fit the calibration data to the following curve types:

- Linear
- Quadratic
- Linear log-log
- Quadratic log-log
- Average response factor (RF)
- Point-to-point
- Cubic spline
- Locally weighted

You can also have LCquan weight the calibration data with the following weighting functions when performing the least squares fit to the calibration data:

- Equal
- 1/X
- $1/X^2$
- 1/Y
- $1/Y^2$
- $1/S^2$, where $S^2 = X^2 + Y^2$

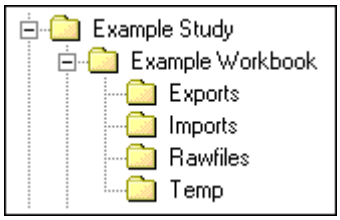
In addition, you can have LCquan ignore the origin, use the origin as a data point, or force the calibration curve to include the origin.

The Quantitate view contains the following pages: Create Method, Create Sequence, Survey, Review All, and Review Reports.

LCquan Folder Structure

When you start a new quantitative analysis project, LCquan creates a hierarchical folder structure for the project on the hard drive. See Figure 8.

Figure 8. Folder structure created by LCquan for a quantitative analysis project



The top-most level in the file structure is the ***study*** folder. This folder is used to organize one or more ***workbook*** folders. Each workbook folder holds all of the information used by LCquan for an individual quantitative analysis project. The workbook folder contains the LCquan file (*.lqn* file), the Instrument Method file(s) (*.meth* file), and the audit log files. In addition, the workbook folder contains the following folders: *Exports*, *Imports*, *Rawfiles*, and *Temp*.

The ***Exports*** folder stores copies of all files that are exported from LCquan, such as report files. The ***Imports*** folder stores a copy of legacy files that you import into the workbook, such as Instrument Method files or sequence files. The ***Rawfiles*** folder contains acquired data files (*.raw* files), as well as any imported raw data files. The ***Temp*** folder contains backup and temporary files used by LCquan.

When you create a new project, LCquan requires you to name the study folder and the workbook folder. You can have as many different workbooks in the same study folder as you want, but each workbook must have a unique name. This is convenient for organizing your quantitative analysis projects. For example, you can use one study folder to organize all of the separate quantitative analysis projects that you are doing for a particular client. You can not rename the subfolders that LCquan creates in the workbook folder.

LCquan manages all of the files associated with the project. You do not need to remember what individual files are named or where they reside on your hard drive. All necessary files are contained within the workbook. In addition, LCquan uses a file tracking feature to maintain a secure file system. Check with your Administrator if you have problems with the security configuration.

IMPORTANT To maintain secure file operations, LCquan tracks the files in the workbook to ensure that none has been modified by an external process. If you modify a file in the workbook by using another software application (including Xcalibur), LCquan will not allow you to reopen the workbook. Therefore, always use LCquan to open and modify the workbook and its associated files.

Acquiring and Processing Data with LCquan

With LCquan, quantitative analysis usually involves the following processes. The order of some of these steps is not rigid. For example, before you acquire data, you can create the Processing Method and Processing Sequence and then use these to define the Acquisition Sequence.

1. Developing an Instrument Method (non-Citrix environment only):

   LCquan uses an Instrument Method to store a specific set of parameters used to operate the autosampler, LC pump or MS pump, mass spectrometer, divert valve, and syringe pump during analysis. See Chapter 2, "Preparing to Perform Quantitative Analysis of Steroids Data."

2. Building an Acquisition Sequence of samples (non-Citrix environment only):

   An Acquisition Sequence defines each sample as a standard, unknown, QC, or blank, and identifies its position on an autosampler tray (if appropriate). See Chapter 2, "Preparing to Perform Quantitative Analysis of Steroids Data."

3. Acquiring data (non-Citrix environment only):

   LCquan allows you to run one sample or a series of samples from the current Acquisition Sequence. See Chapter 2, "Preparing to Perform Quantitative Analysis of Steroids Data."

4. Exploring your data:

   The Explore view is a "sandbox" where you can open a raw data file and play with peak detection and integration parameters that you will use in the Quantitate view. See Chapter 3, "Exploring Your Data."

5. Creating a Processing Method:

   LCquan uses a Processing Method to identify, detect, and integrate components in a chromatogram, generate calibration curves, quantify unknowns, and produce reports. See Chapter 4, "Creating a Processing Method."

6. Building a Processing Sequence:

   A Processing Sequence consists of a list of sample data files and includes information on sample type and calibration or QC level. See Chapter 5, "Creating a Processing Sequence."

7. Processing the raw files and reviewing the calibration and quantitative analysis results:

   After LCquan processes the raw data file, you can evaluate and rework the calibration standards by modifying the peak detection and integration settings. You can also integrate peaks manually. See Chapter 6, "Processing the Raw Files and Reviewing the Analytical Results."

8. Creating reports:

   LCquan provides both standard and custom printed reports. Standard reports contain preselected methods and results summaries. Custom reports contain methods and results summaries that you select from a list of options. See Chapter 6, "Processing the Raw Files and Reviewing the Analytical Results."

Using This Manual

This manual contains a stepwise procedure for performing quantitative analysis on an example data set provided in the *Xcalibur\examples\data* folder of your Xcalibur data system. This data was collected on a steroids mixture in the applications laboratories at Thermo Scientific by using LC/MS/MS techniques in the APCI ionization mode.

The steroids mixture contains hydrocortisone, deoxycorticosterone, and progesterone as the target compounds. A fourth steroid, methyltestosterone, is used as an internal standard (ISTD). The amount of the internal standard in the calibration standards in this example is 0.63 ng. Calibration standards were prepared to give five calibration levels with amounts of 0.63, 1.25, 2.5, 5.0, and 10.0 ng. This example also includes five unknown samples. No QC samples are included.

This manual leads you through the steps of quantitative analysis of the steroids data set, including importing the existing steroids raw data files into a new workbook folder, creating a new Acquisition Sequence, Processing Method and Processing Sequence, processing the raw data files, reviewing calibration standards and unknowns, and creating and reviewing reports. Although you do not need to create an Instrument Method or an Acquisition Sequence to follow the quantitative analysis procedure for steroids, procedures on how to do this are also included in this manual.

We recommended that you follow the steroids quantitative analysis in the following order:

1. "Creating a New Study and Workbook" on page 22
2. "Creating an Acquisition Sequence for the Steroids Example" on page 30
3. "Editing the Acquisition Sequence for the Steroids Example" on page 34
4. "Generating a Peak List" on page 54
5. "Creating a Processing Method" on page 71
6. "Creating a Processing Sequence" on page 87
7. "Processing the Raw Files" on page 100
8. "Reviewing the Calibration Curve and QCs" on page 100
9. "Reviewing All Results" on page 112

Note Sections of this manual that provide only additional information and are not necessary for the steroids quantitative analysis are clearly marked. You can skip these sections while performing the analysis; however, they provide important information and should be read at a later time.

LCquan for Citrix

LCquan supports Citrix Presentation Server™. Citrix Presentation Server is used for application virtualization to manage the LCquan software configuration and maintenance. The LCquan virtual application runs on centralized servers; only keystrokes and mouse clicks travel over the network from the user's computer, and only screen images travel over the network to the user's computer. You need to validate LCquan only on the server.

LCquan 2.5 SP1 and later versions support the Citrix environment for data processing. LCquan for Citrix includes only the Explore and Quantitate views of LCquan. Certain actions within LCquan, such as data acquisition, can be done only on a local, validated computer and not the server.

Preparing to Perform Quantitative Analysis of Steroids Data

This chapter describes how to start LCquan and create a new study and workbook in preparation for analyzing pre-existing steroids data. This chapter also describes the Instrument Setup view and the Acquisition view of LCquan. Although the step-by-step procedure for performing quantitative analysis contained in this manual does not require that you create Instrument Methods or acquire your own data, this chapter outlines the procedures for doing this.

Contents

- Creating a New Study and Workbook
- Reviewing the Instrument Setup View
- Reviewing the Acquisition View

Creating a New Study and Workbook

When you start LCquan, you can open an existing study and workbook, or you can create a new study and workbook set. You can also choose to open a workbook in review mode. In review mode you can analyze existing data and create methods, but you cannot acquire data.

For the quantitative analysis of steroids data, you create a new study and workbook and import raw steroids data to start your analysis.

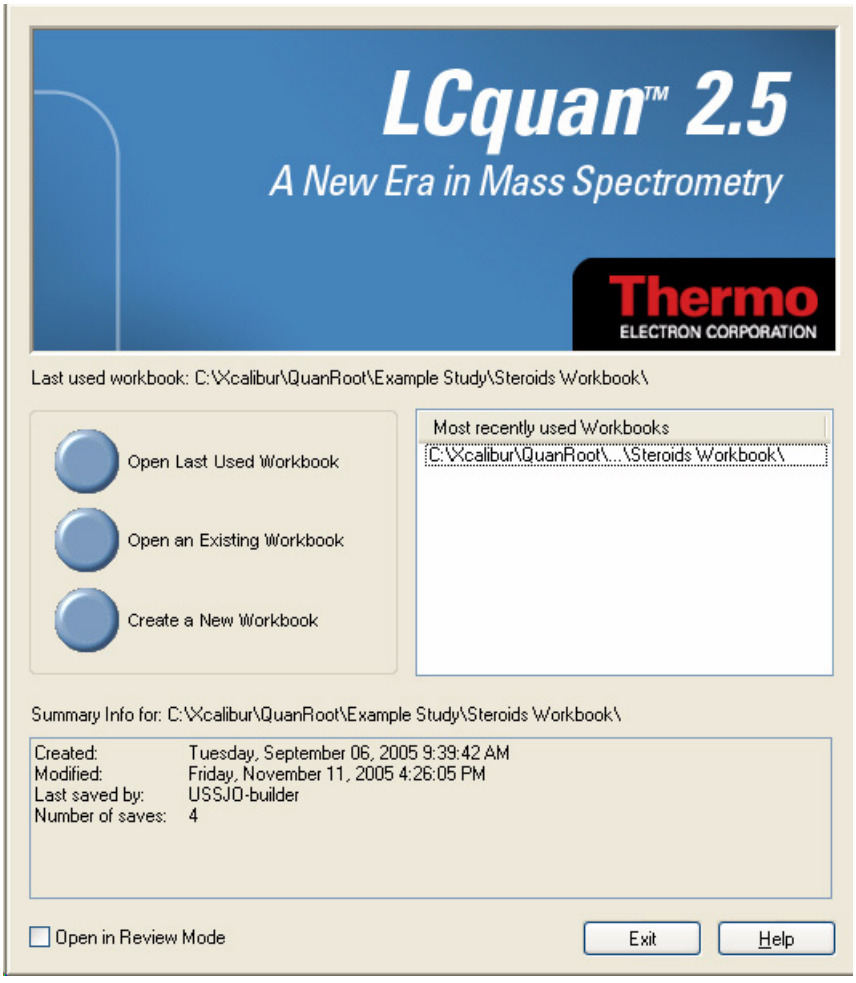
❖ To create a new study and workbook

1. Open LCquan from your desktop by choosing **Start > Programs > Xcalibur > LCquan**.

   LCquan displays a startup dialog box from which you can open the last used workbook, open an existing workbook, or create a new workbook. See Figure 9.

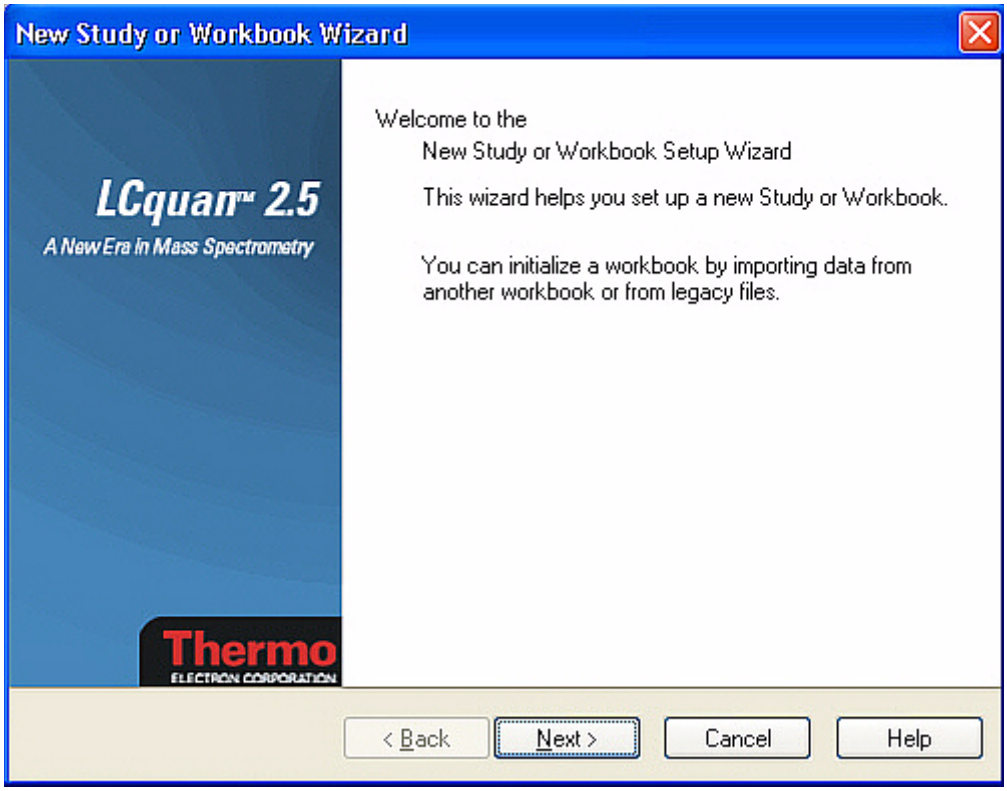
   If you have used LCquan previously, the name of the most recently opened workbook appears directly above the buttons. In addition, the Most Recently Used Workbooks list displays up to eight workbooks; you can open a workbook from this list by clicking on it.

Figure 9. Startup dialog box for LCquan

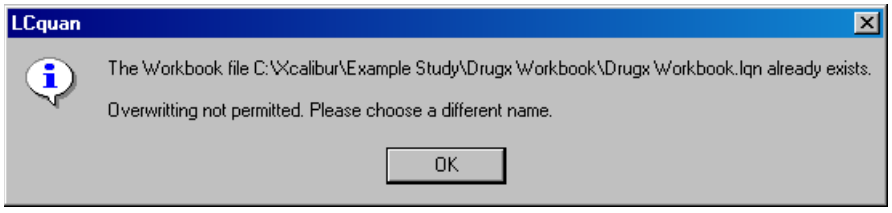


2. Ensure that the **Open in Review Mode** check box is **cleared**.
3. Click on the **Create New Study or Workbook** button. The Welcome page of the New Study or Workbook Wizard appears. See Figure 10.
4. Click on **Next** to continue.
5. Name the study and workbook folders:
   a. In the Study Name text box, type **Practice Study**.
   b. In the Workbook Name text box, type **Steroids Workbook**.
   c. Click on **Next** to continue.

Figure 10. New Study or Workbook Wizard – Welcome page

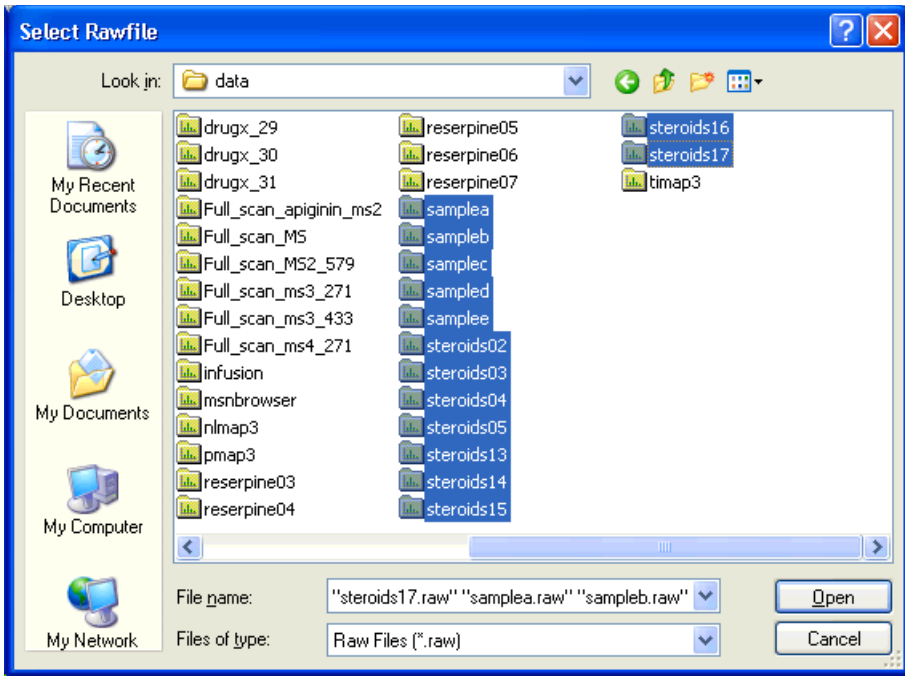

New Study or Workbook Wizard
LCquan™ 2.5
A New Era in Mass Spectrometry
Thermo
ELECTRON CORPORATION
Welcome to the
New Study or Workbook Setup Wizard
This wizard helps you set up a new Study or Workbook.
You can initialize a workbook by importing data from another workbook or from legacy files.
< Back
Next >
Cancel
Help

Note LCquan does not permit you to overwrite existing data. If you attempt to name a new study and workbook set the same as an existing set, LCquan displays the following message box. Click OK, and then type another study or workbook name.


LCquan
The Workbook file C:\Xcalibur\Example Study\Drugx Workbook\Drugx Workbook.lqn already exists.
Overwritting not permitted. Please choose a different name.
OK

6. Select the raw data files to import into the new workbook:
   a. Select the **Import Rawfiles** check box.
   b. Click on **Browse** to open the Select Rawfile dialog box.
   c. Browse to **Xcalibur\examples\data**.
   d. Click on ***samplea***, and then hold down SHIFT and click on ***steroids17*** to select the 14 steroid raw files. See Figure 11.
   e. Click on **Open** and then **Next** to continue.

Figure 11. Select Rawfile dialog box, showing the selected steroids raw files



7. Ensure that the **Import Instrument Method, Acquisition Sequence,** and **Processing Parameters** check box is **cleared**, and click on **Next**.
8. Click on **Finish**. LCquan creates the new study and workbook folders and imports the raw data files into the *Rawfiles* subfolder.

After you create a new workbook, LCquan opens the Instrument Setup view. Because there are no Instrument Methods for the configured devices yet, LCquan creates default methods.

You use the raw files that you just imported to complete the steroids quantitative analysis described in this manual. Therefore, you do not need to create an Instrument Method or acquire your own data. If you want to continue directly with the quantitative analysis of steroids procedure, skip the next several sections in this chapter and continue with "Creating an Acquisition Sequence for the Steroids Example" on page 30.

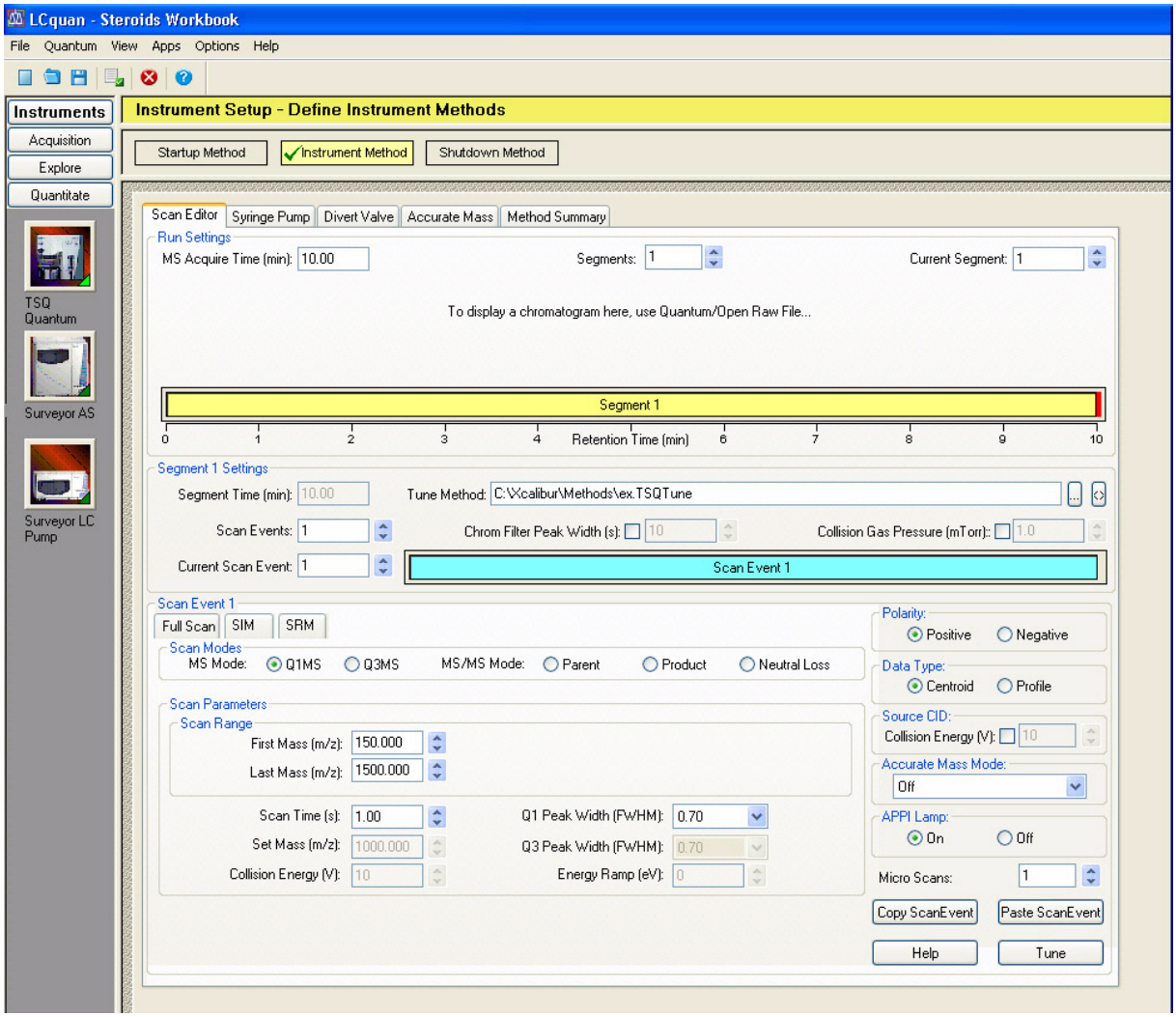
Reviewing the Instrument Setup View

The Instrument Setup view allows you to define a set of experimental parameters for each configured instrument. These parameters include the operating settings for the instrument (collectively referred to as the ***Instrument Method***), as well as any optional instrument settings to be used before or after LCquan runs a sequence of samples (***Startup Method*** and ***Shutdown Method***, respectively). The parameters that are displayed in the Instrument Setup view depend on the instrument that you select in the viewbar on the left side of the view. See Figure 12.

Note

- The Instrument Setup view is not available in LCquan for Citrix.
- For this example, if you do not create an Acquisition Sequence, you need to create a Processing Sequence later.

Figure 12. Instrument Setup view, showing the default Instrument Method for TSQ Quantum



This section contains the following topics:

- Creating Instrument Methods
- Importing an Existing Instrument Method

Creating Instrument Methods

Although you do not need to create Instrument Methods to follow the quantitative analysis procedure described in this manual, the general procedure is outlined below. For specific information on how to set up a particular instrument, refer to the online Help.

❖ To create Instrument Methods for your configured instruments

1. Select an instrument by clicking on its icon in the viewbar.

   LCquan displays one or more pages of instrument parameters.

   Note You must configure the instrument before you can define an Instrument Method. If you need to configure an instrument, you must first close LCquan and Xcalibur and choose **Start > Programs > Xcalibur > Instrument Configuration** to open the Instrument Configuration dialog box.

2. Ensure that the **Instrument Method**

button is selected.

3. Enter the instrument settings that are appropriate for the experiment that you want to perform. Note that the Instrument Setup view might contain multiple pages or dialog boxes.

4. Repeat steps 1 through 3 for each instrument that you want to set up.

5. If you want to create an optional Startup Method that LCquan will run before it runs the first sample in an acquisition, do the following:

   a. Select the appropriate instrument by clicking on its icon in the viewbar.

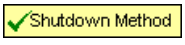
   b. Click on the **Startup Method**

button.

   c. Enter the startup settings for the instrument.

6. If you want to create an optional Shutdown Method that LCquan will run after it runs the last sample in an acquisition, do the following:

   a. Select the appropriate instrument by clicking on its icon in the viewbar.

   b. Click on the **Shutdown Method** 

button.

   c. Enter the shutdown settings for the instrument.

If you create or modify an Instrument Method, LCquan automatically saves it when you leave the Instrument Setup view. The file name is based on the workbook name (for example, *Steroids Workbook.meth*).

After a method has been saved in the workbook, it remains part of the workbook even if the configured instrument is removed.

Importing an Existing Instrument Method

LCquan also lets you import an existing Instrument Method into your current workbook instead of creating a new method. You can use the method as is or edit it, if necessary.

You can import an Instrument Method in the following ways:

- New Study Wizard. When you create a new workbook, the New Study Wizard gives you the option of importing Instrument Methods, Acquisition Sequences, and processing parameters to initialize the new workbook. You can select to import all of this data from an existing workbook, or you can select to import an Instrument Method from an individual legacy file (*.meth* file).
- Instrument Setup view menu command.
- Instrument Method File dialog box. Choose **File > Import Instrument Method** to display the Import Instrument Method File dialog box. This dialog box allows you to import an Instrument Method from an individual legacy file (*.meth* file).

Reviewing the Acquisition View

The Acquisition view allows you to define an Acquisition Sequence, run individual samples and sequences, and monitor real-time data acquisition. This view is made up of the following components:

- Setup Sequence Page
- Run Sequence Dialog Box
- Naming Components and Assigning Calibration Levels
- Acquisition View – Status Page

You use the viewbar on the left side of the view to select the page or dialog box.

Note You have already imported existing raw data into your workbook. You do not need to acquire your own data to follow the quantitative analysis procedure described in this manual.

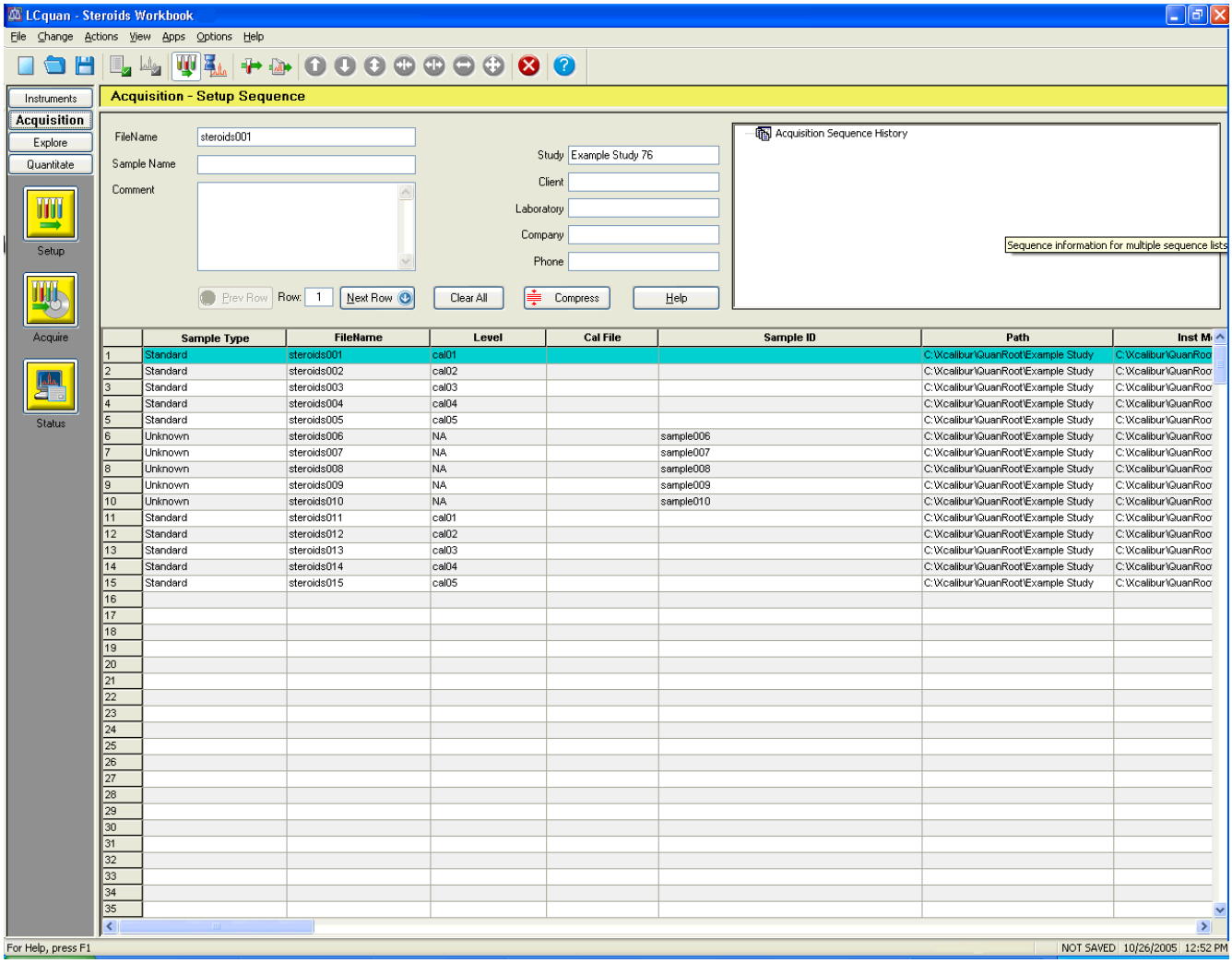
Setup Sequence Page

The Acquisition view – Setup Sequence page allows you to create or modify an Acquisition Sequence. It includes (clockwise from the top left) the Sequence Header, the Acquisition Sequence History pane, and the Sequence table. See Figure 13.

The ***Acquisition Sequence*** is a list of samples and defined settings that LCquan uses to acquire data. The Acquisition Sequence can contain unknown samples, calibration standard samples, quality control (QC) samples, and blank samples. Each sample can be defined by some or all of the following settings:

- Sample type
- File name
- Sample identification
- QC or Standard level
- Internal standard correction amount
- Dilution factor
- Vial position
- Injection volume
- Sample volume
- Sample weight
- Sample name

Figure 13. Acquisition view – Setup Sequence page, showing an Acquisition Sequence


LCquan - Steroids Workbook
File Change Actions View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Setup
Acquire
Status
Acquisition - Setup Sequence
FileName steroids001
Sample Name
Comment
Study Example Study 76
Client
Laboratory
Company
Phone
Acquisition Sequence History
Sequence information for multiple sequence lists
Prev Row
Row: 1
Next Row
Clear All
Compress
Help
Sample Type FileName Level Cal File Sample ID Path Inst M
1 Standard steroids001 cal01 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
2 Standard steroids002 cal02 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
3 Standard steroids003 cal03 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
4 Standard steroids004 cal04 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
5 Standard steroids005 cal05 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
6 Unknown steroids006 NA sample006 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
7 Unknown steroids007 NA sample007 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
8 Unknown steroids008 NA sample008 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
9 Unknown steroids009 NA sample009 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
10 Unknown steroids010 NA sample010 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
11 Standard steroids011 cal01 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
12 Standard steroids012 cal02 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
13 Standard steroids013 cal03 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
14 Standard steroids014 cal04 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
15 Standard steroids015 cal05 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
For Help, press F1
NOT SAVED 10/26/2005 12:52 PM

Each row of the sequence corresponds to one sample injection. You need an Acquisition Sequence to acquire data and a Processing Sequence to process data. The Acquisition Sequence and the Processing Sequence can be the same. Each sequence has a limit of 5000 samples.

The following subtopics describe how to use the Setup Sequence page:

- Creating an Acquisition Sequence for the Steroids Example
- Editing the Acquisition Sequence for the Steroids Example
- Importing or Copying an Existing Sequence
- Editing an Acquisition Sequence
- Assigning Calibration or QC Levels
- Printing and Exporting the Acquisition Sequence

Creating an Acquisition Sequence for the Steroids Example

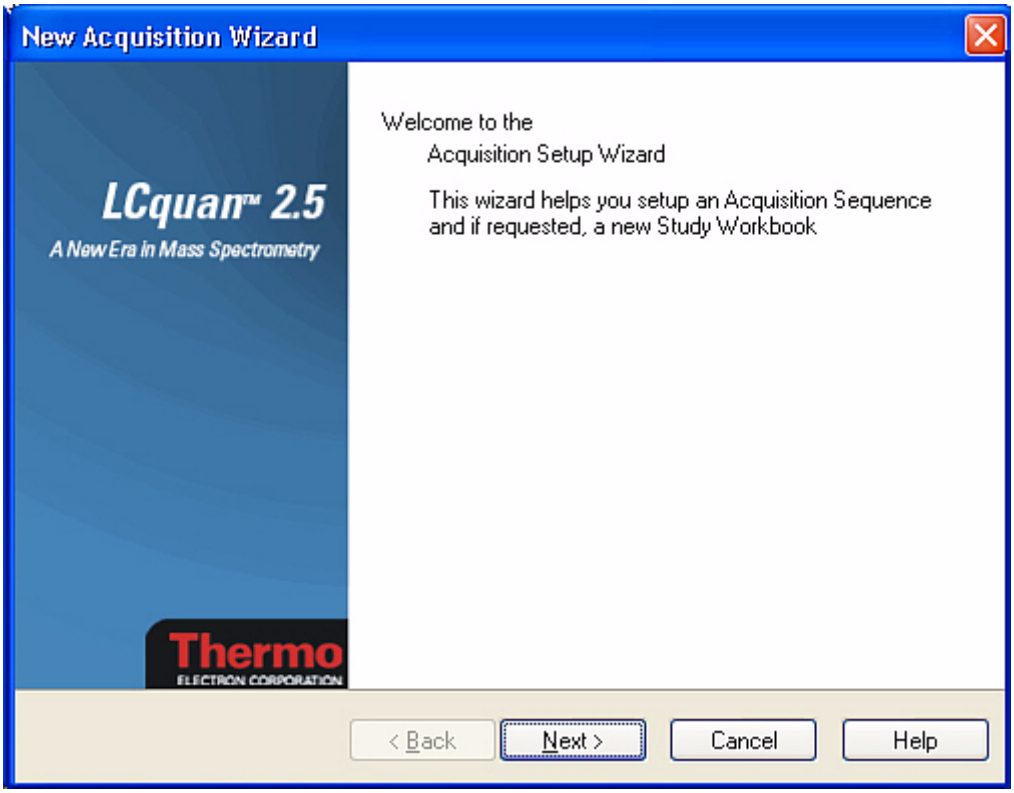
Although you do not need to create an Acquisition Sequence to follow the quantitative analysis procedure described in this manual, the procedure is outlined below. For more information, refer to the online Help.

❖ To define an Acquisition Sequence for the steroids example

1. From the Instrument Setup view (see previous section), click on the **Acquisition** Acquisition button in the viewbar. If no Acquisition Sequence is defined in the workbook, the New Acquisition Wizard appears. See Figure 14.

   Tip If the New Acquisition Wizard does not appear, right-click on the Sequence table and choose **New Acquisition Sequence Wizard** from the shortcut menu.

   Figure 14. New Acquisition Wizard – Welcome page



2. Click on **Next** to continue.
3. Specify not to create a new workbook for the acquisition:

   Ensure that the **Create a New Workbook** check box is **cleared** and click on **Next**.

   Note If you want to create a new workbook, select the **Create a New Workbook** check box and click on **Next**. Then, provide a name for the new study and workbook and click on **Next**.

4. Specify not to import an existing sequence into the workbook:

   Ensure that the **Base Sequence On a Previously Saved File** check box is **cleared** and click on **Next**.

   Note If you want to import a sequence, select the **Base Sequence On a Previously Saved File** check box. Then, click on **Browse** and locate the sequence file (file with extension *.sld*, *.ipp*, *.csv*) or the LCquan file (*.lqn*) that contains the sequence. (The sequence for the steroids example is *C:\Xcalibur\examples\method\steroids.sld*.) You can always edit the sequence after you import it. Click on **Next**. Go to step 12.

5. Specify the raw file names:

   - Base File Name: **steroids**
   - Starting Number: **1**

   Click on **Next**.

   LCquan appends a three-digit number (001-999) to the base file name so that each sample in a sequence has a unique identification.

6. Specify the unknown sample information:

   - Number of Samples: **5**
   - Injections per sample: **1**
   - Base Sample ID: **sample**

   Click on **Next**.

   LCquan generates sample IDs that uniquely identify the unknown samples in the sequence by appending numerical values to a base sample ID.

7. Specify the autosampler settings:

   - Initial Vial Position: **A:1**
   - Re-Use Vial Positions (for replicate samples):
   - Injection Volume Obtained from Autosampler Method:

   Click on **Next**.

8. Specify the calibration standard information:
   - Add Standards:
   - Based on Automatically Generated Cal Levels:
   - Number of Cal Levels: **5**
   - Cal Level Base Name: **cal**
   - Number of Injections Per Level: **2**
   - Add Blanks:
   - Fill in Sample ID for Standards:

   Click on **Next**.

   LCquan generates names for the levels by appending numerical values to the base name.

   Note

   - You can use the calibration levels and component names from the Processing Method (if one exists in the workbook). Refer to "Importing the Components and Levels into the Acquisition Sequence" on page 84.
   - The steroids data set does not contain any quality control or blank samples. When a data set does contain QCs or blanks, you associate the raw data files to the QC or blank sample type in the same manner as above.

9. Specify that there are no QCs:
   a. Add QCs:
   b. Click on **Next**.

   You can use the QC levels from the Processing Method (if one exists in the workbook) or you can have LCquan generate names for the levels by appending numerical values to a base name.

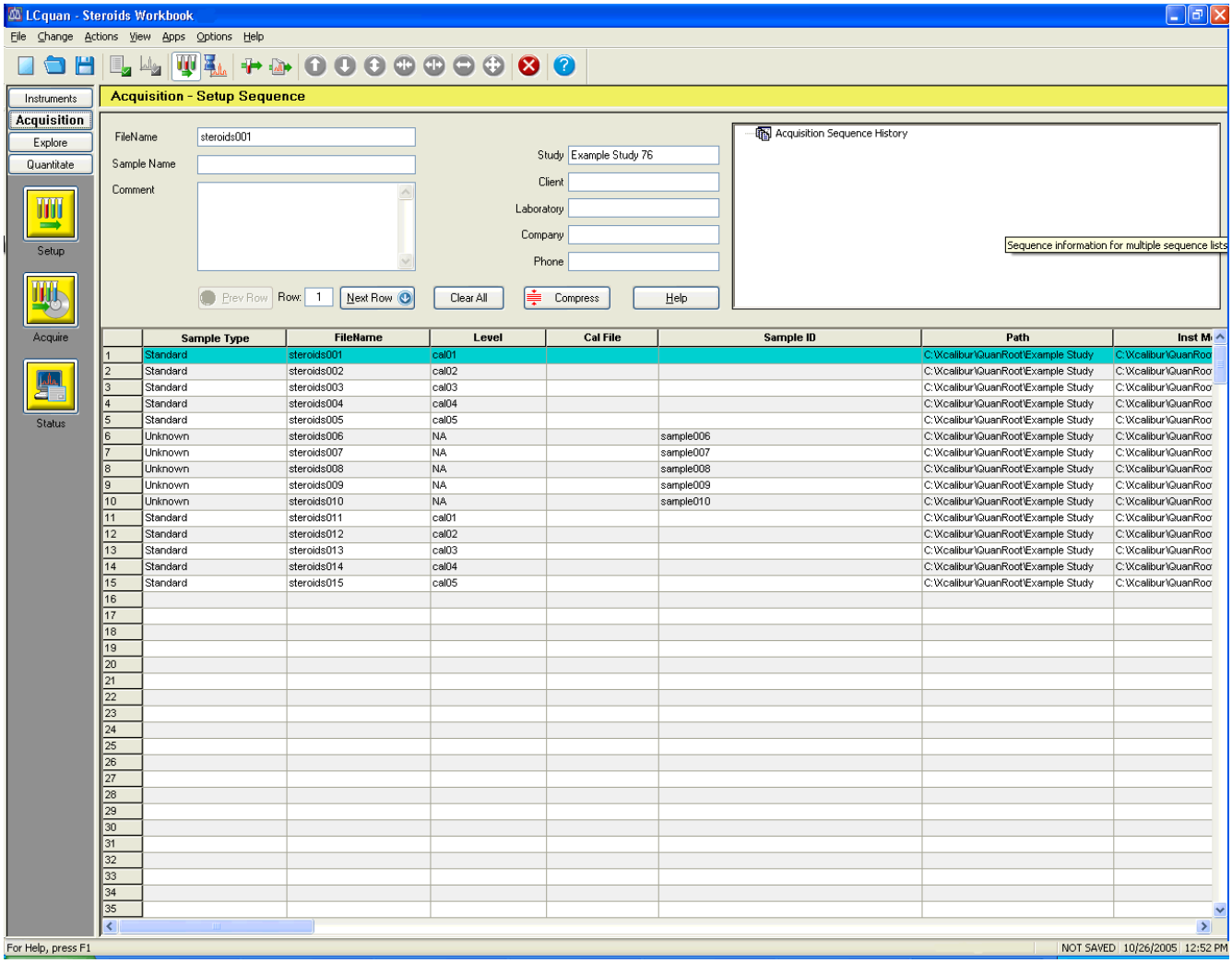
10. Specify temporary component names for the steroids:
    - Number of Components: **4**
    - Component Base Name: **Component**

    Click on **Next**.

11. Accept the default labels and the values for the five user-defined text boxes that appear in the Sequence Header area of the page. (The information that you enter in these text boxes appears in the header of the raw file.) Click on **Next**.

12. Click on **Finish** to close the wizard. LCquan displays the Acquisition view – Setup Sequence page. See Figure 15.

Figure 15. Acquisition view – Setup Sequence page


LCquan - Steroids Workbook
File Change Actions View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Setup
Acquire
Status
Acquisition - Setup Sequence
FileName steroids001
Sample Name
Comment
Study Example Study 76
Client
Laboratory
Company
Phone
Acquisition Sequence History
Sequence information for multiple sequence lists
Prev Row
Row: 1
Next Row
Clear All
Compress
Help
Sample Type FileName Level Cal File Sample ID Path Inst M
1 Standard steroids001 cal01 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
2 Standard steroids002 cal02 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
3 Standard steroids003 cal03 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
4 Standard steroids004 cal04 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
5 Standard steroids005 cal05 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
6 Unknown steroids006 NA sample006 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
7 Unknown steroids007 NA sample007 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
8 Unknown steroids008 NA sample008 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
9 Unknown steroids009 NA sample009 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
10 Unknown steroids010 NA sample010 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
11 Standard steroids011 cal01 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
12 Standard steroids012 cal02 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
13 Standard steroids013 cal03 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
14 Standard steroids014 cal04 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
15 Standard steroids015 cal05 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
For Help, press F1
NOT SAVED 10/26/2005 12:52 PM

Editing the Acquisition Sequence for the Steroids Example

Although you are not acquiring data in this example, in this section you edit the Acquisition Sequence as if you were. This Acquisition Sequence would produce the raw files in the steroids data set. Later, you can use this Acquisition Sequence as the Processing Sequence.

❖ To edit the Acquisition Sequence

1. Delete rows 1 and 15:
   a. Select row 1 by clicking on the **1** at the left of the row.
   b. Delete row 1 by right-clicking on row 1, and then selecting **Delete Rows** from the shortcut menu.
   c. Select row 15 by clicking on the **15** at the left of the row.
   d. Delete row 15 by right-clicking on row 15, and then selecting **Delete Rows** from the shortcut menu.
   e. Click on **Compress**

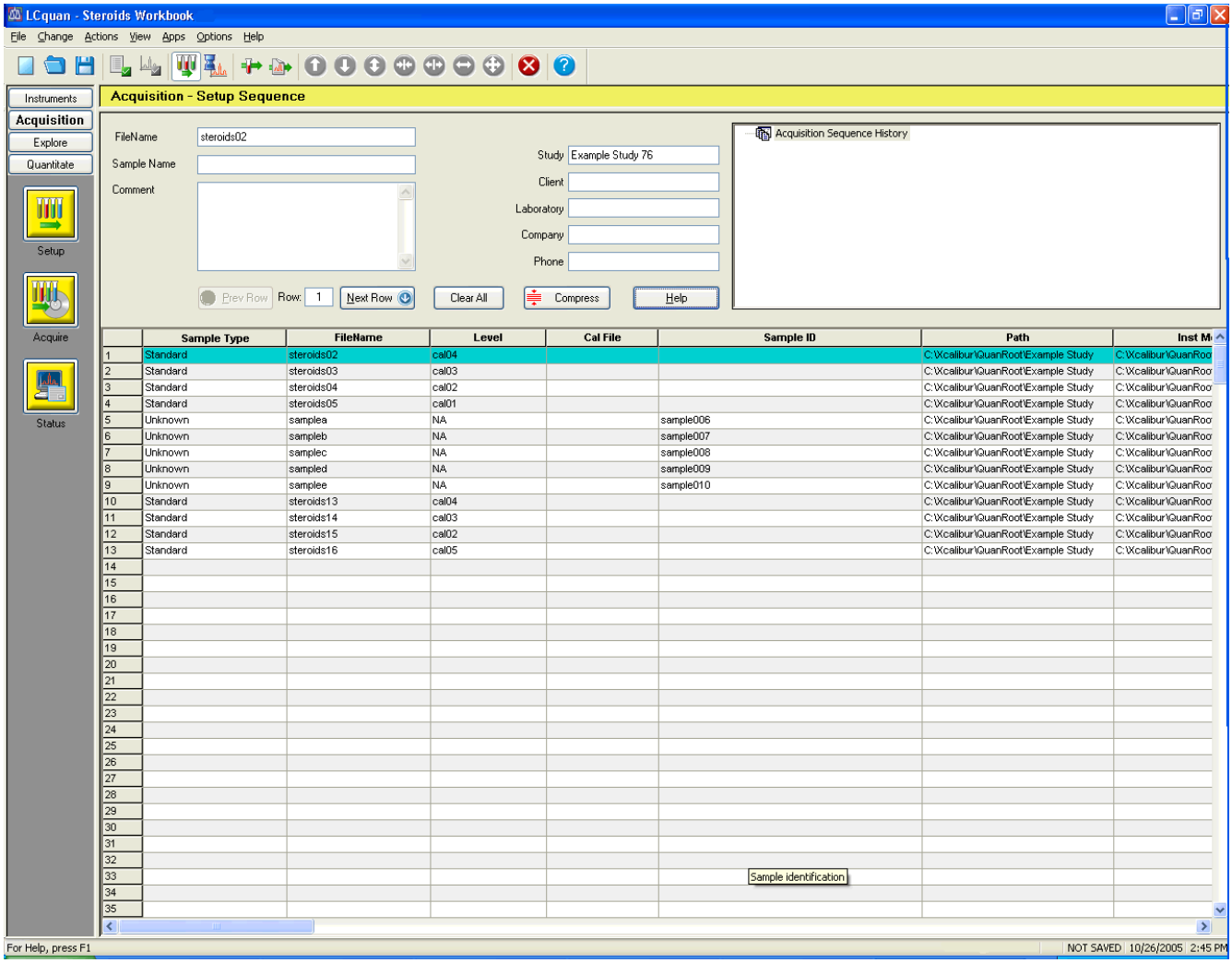
to remove blank rows.
2. Change the file names and calibration levels as shown in Table 1. You can change a file name or calibration level by clicking in the box and then using the down arrow.

Table 1. Sample types, file names, and calibration levels in the Acquisition Sequence for the steroids example

#	Sample Type	File Name	Level
1	Standard	steroids02	cal04
2	Standard	steroids03	cal03
3	Standard	steroids04	cal02
4	Standard	steroids05	cal01
5	Unknown	samplea	
6	Unknown	sampleb	
7	Unknown	samplec	
8	Unknown	sampled	
9	Unknown	samplee	
10	Standard	steroids13	cal04
11	Standard	steroids14	cal03
12	Standard	steroids15	cal02
13	Standard	steroids16	cal05

The Acquisition Sequence should now look like Figure 16.

Figure 16. Edited Acquisition Sequence for the steroids example


LCquan - Steroids Workbook
File Change Actions View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Setup
Acquire
Status
Acquisition - Setup Sequence
FileName steroids02
Sample Name
Comment
Study Example Study 76
Client
Laboratory
Company
Phone
Acquisition Sequence History
Prev Row
Row: 1
Next Row
Clear All
Compress
Help
Sample Type FileName Level Cal File Sample ID Path Inst M
1 Standard steroids02 cal04 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
2 Standard steroids03 cal03 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
3 Standard steroids04 cal02 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
4 Standard steroids05 cal01 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
5 Unknown samplea NA sample006 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
6 Unknown sampleb NA sample007 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
7 Unknown samplec NA sample008 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
8 Unknown sampled NA sample009 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
9 Unknown samplee NA sample010 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
10 Standard steroids13 cal04 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
11 Standard steroids14 cal03 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
12 Standard steroids15 cal02 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
13 Standard steroids16 cal05 C:\Xcalibur\QuanRoot\Example Study C:\Xcalibur\QuanRoo
Sample identification
For Help, press F1
NOT SAVED 10/26/2005 2:45 PM

3. Choose **File > Save** to save the Acquisition Sequence and workbook.

To continue with the steroids example, continue with the next topic: "Naming Components and Assigning Calibration Levels."

Naming Components and Assigning Calibration Levels

You can change the component names and define or modify the calibration levels and QC levels that the Acquisition Sequence uses by using the Acquisition Levels dialog box:

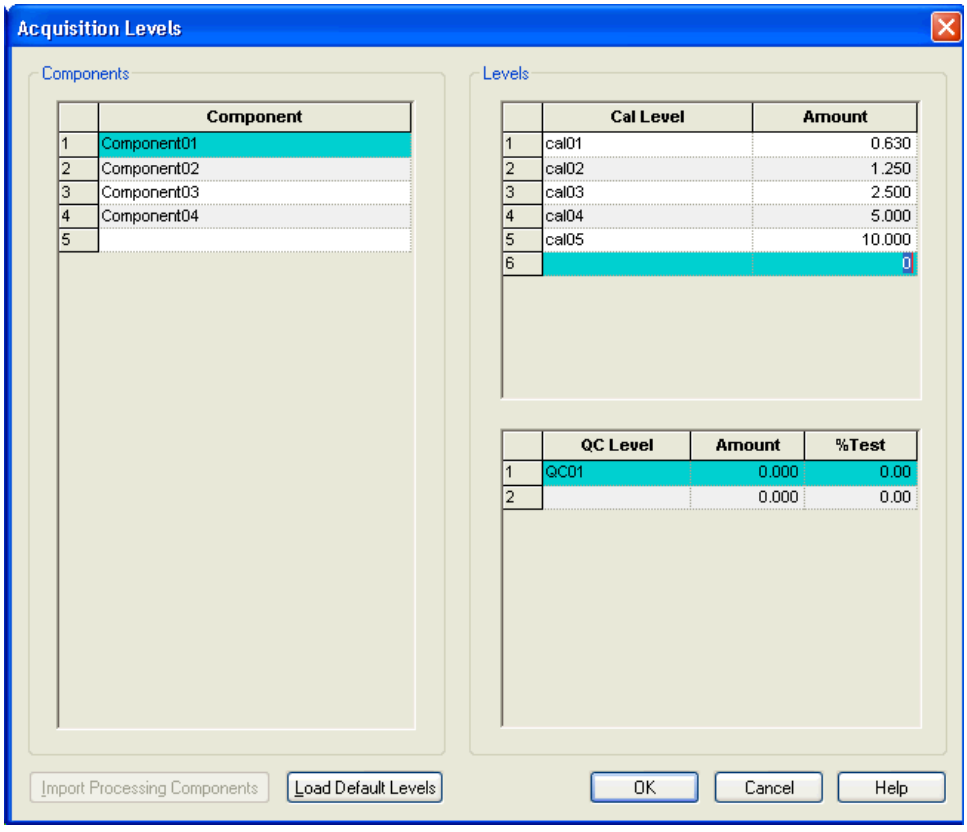
❖ **To change the component names and define or modify the calibration levels and QC levels**

1. Right-click in the Sequence table and choose **Standard and QC Levels** from the shortcut menu to open the Acquisition Levels dialog box.
2. Modify the Calibration Levels table as shown in Table 2 and Figure 18.

Table 2. Calibration levels

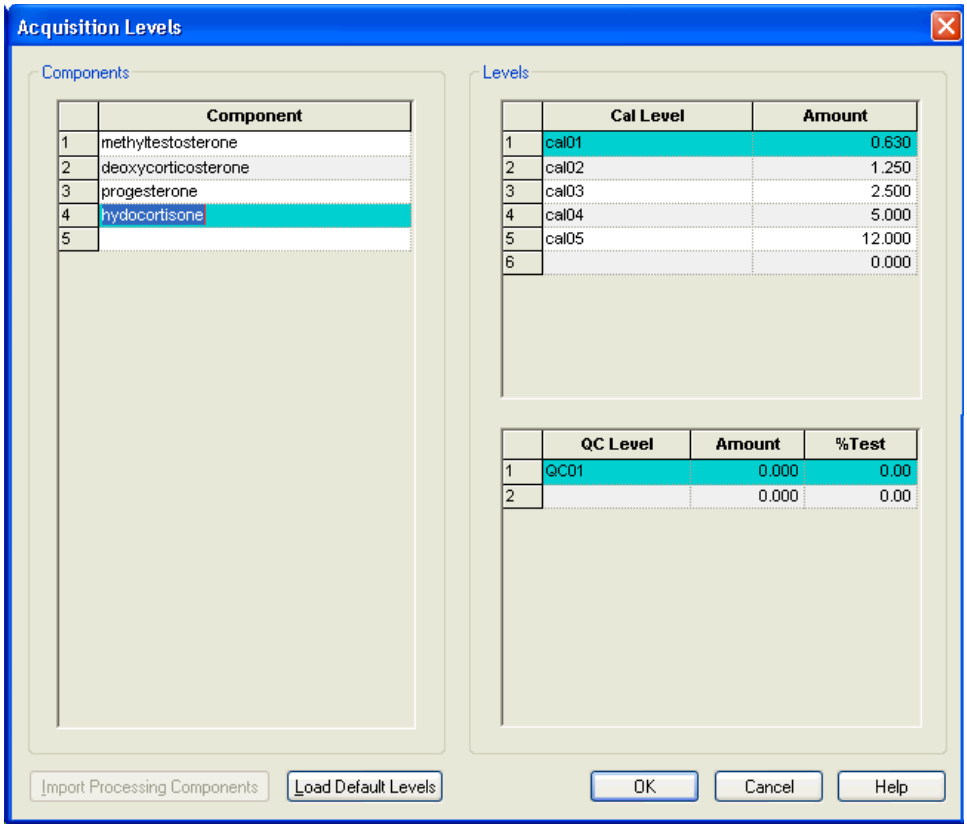
Calibration Level	Amount (ng)
cal01	0.63
cal02	1.25
cal03	2.50
cal04	5.00
cal05	10.00

Figure 17. Acquisition Levels dialog box with initial settings



3. Click on row **6** in the Calibration Levels table.
4. Right-click on the Calibration Levels table and choose **Copy Current Component Levels to all Target Compounds** from the shortcut menu.
5. Change the component names by selecting the existing name in the Components table and then typing the new name:
   a. Change **Component1** to **methyltestosterone**.
   b. Change **Component2** to **deoxycorticosterone**.
   c. Change **Component3** to **progesterone**.
   d. Change **Component4** to **hydrocortisone**.
   e. Click on Row **5** in the Components table
6. Click on **hydrocortisone** in the Component List and change the cal05 level to **12**. The Acquisition Levels dialog box should look like Figure 19.
7. Click on **OK**.

Figure 18. Acquisition Levels dialog box, showing components and calibration levels for the steroids example



To continue with the steroids example, go on to Chapter 3, "Exploring Your Data." For more information on acquiring data and Acquisition Sequences, continue with the next topic: "Importing or Copying an Existing Sequence".

Importing or Copying an Existing Sequence

In some cases, instead of creating a new Acquisition Sequence, it might be more convenient to use an existing sequence as your Acquisition Sequence. You can do this by importing an existing Acquisition Sequence or by copying the Processing Sequence from your current workbook.

You can import or copy an existing sequence in the following ways:

- By using the New Study Wizard:

  When you create a new workbook, the New Study Wizard gives you the option of importing Instrument Methods, Acquisition Sequences, and processing parameters to initialize the new workbook. You can select to import all of this data from an existing workbook, or you can select to import an Acquisition Sequence from an individual legacy file (*.sld, .ipp,* or *.csv* file) or from an LCquan file (*.lqn* file).

  If you import an Acquisition Sequence from an individual legacy file into a workbook that contains a Processing Method but does not contain a Processing Sequence, LCquan uses the same sequence for both.

  Note When the imported Acquisition Sequence is also used as the Processing Sequence, the level names must match those defined in the Processing Method:

  If the calibration or quality control level names in the sequence are different from those in the Processing Method, LCquan displays the Standard Level Names Association dialog box of the QC Level Names Association dialog box. You can use these dialog boxes to associate the level names in the sequence with the level names in the Processing Method.

  If the imported sequence contains calibration or QC levels but the Processing Method does not, LCquan displays a warning box to inform you. It then displays the Sample Type Assignment dialog box. You can select to change the sample type of these samples to unknown in the Processing Sequence or to discard the samples that have these levels in the Processing Sequence.

- By using the New Acquisition Sequence Wizard in the Acquisition view:

  When you use the New Acquisition Sequence Wizard in the Acquisition view, you have the option to import a previously saved file. You can import a sequence from an individual legacy file (*.sld, .ipp,* or *.csv* file) or from an LCquan file (*.lqn* file).

- By using menu commands from within the Acquisition view:

  From the Setup Sequence page of the Acquisition view, you can choose **File > Import Acquisition Sequence > From File** to display the Import Sequence File dialog box. This dialog box allows you to import a sequence from an individual legacy file (*.sld, .ipp*, or *.csv* file) or from an LCquan file (*.lqn* file).

You can also right-click anywhere within the page and choose **Import Acquisition Sequence > From File** from the shortcut menu to display the Import Sequence File dialog box.

- By dragging and dropping sequences from the Acquisition Sequence History pane:

  The Acquisition Sequence History lists every Acquisition Sequence that was submitted to the acquisition queue in the life of the workbook. You can select one or more of these sequences and then drag and drop them into the Sequence table. Hold down the CTRL key to select multiple sequences.

- By copying the Processing Sequence if one already exists in the current workbook:

  From the Setup Sequence page of the Acquisition view, you can choose **File > Import Acquisition Sequence > Copy From Processing Sequence** to copy the Processing Sequence from the current workbook into the Acquisition Sequence.

  You can also right-click anywhere within the page and choose **Import Acquisition Sequence > Copy From Processing Sequence** from the shortcut menu to copy the Processing Sequence.

Editing an Acquisition Sequence

After you create or import an Acquisition Sequence, you can edit it by using the Acquisition view – Setup Sequence page. You can change the Acquisition Sequence in the following ways:

- By editing it directly
- By modifying its arrangement
- By changing the user labels that appear in the Sequence Header

Editing the Acquisition Sequence Directly

You can edit the Acquisition Sequence by manually changing the entry in any of the cells in the Sequence table. To change the sample type or the calibration or QC level, select a new option from the list box in the Sequence table. For example, to change the sample type for a particular sample, click in the cell in the Sample Type column, click on the arrow, and then select the new sample type from the list. To change any other entry, simply type in the appropriate text box. For example, to change the file name for a particular sample, click in the cell in the File Name column, delete the old name, and type the new name.

In addition, instead of typing the injection volume for a sample, you can specify that LCquan is to use the injection volume from the Instrument Method for the autosampler. To do this, select a sample row (or rows) and then right-click anywhere in the Sequence table to display the shortcut menu. Choose **Get Injection Volume From AS**. In the rows that you selected, the cells in the Injection Volume column now display *From AS*.

You can change a range of cells by using the Sequence Fill Down dialog box. This dialog box allows you to fill selected rows of selected columns with duplicate text entries or appropriately sequenced number entries. Right-click in the sequence to display a shortcut menu. Then, choose **Fill Down** to open the Sequence Fill Down dialog box.

You can also use common keyboard shortcuts to edit the sequence: CTRL+C copies the selected row, CTRL+V pastes a copied row, and DELETE removes the selected row from the sequence. Finally, the shortcut menu, which is displayed by right-clicking in the Sequence table, contains commands that allow you to delete rows, to insert rows, or to duplicate rows.

Modifying the Arrangement of the Acquisition Sequence

You can modify the arrangement of the Acquisition Sequence by using shortcut menu commands to change the sorting order of the samples or to change the columns that are displayed in the Sequence table. Right-click in the Sequence table to display the shortcut menu. Refer to the online Help for information on each of the commands.

In addition, you can rearrange the order of the Acquisition Sequence by clicking on and dragging a row to move it. To do this, click on the row number in the left-most column of the Sequence table and continue to hold down the mouse button. Then, drag the selected row up or down in the table. A red horizontal line appears to indicate the new position of the row in the table. (The row will be pasted into the table row below the red line.) Release the mouse button to paste the row into the new position. If pasting the row will overwrite an existing row, a message box appears to warn you. Click on **Yes** or **No** as appropriate.

To remove empty rows from the Acquisition Sequence, click on **Compress**.

Changing the User Labels and Values

You can change the labels and the values for the five user-defined text boxes that appear in the Sequence Header area of the page. (The information that you enter in these text boxes appears in the header of the raw file.)

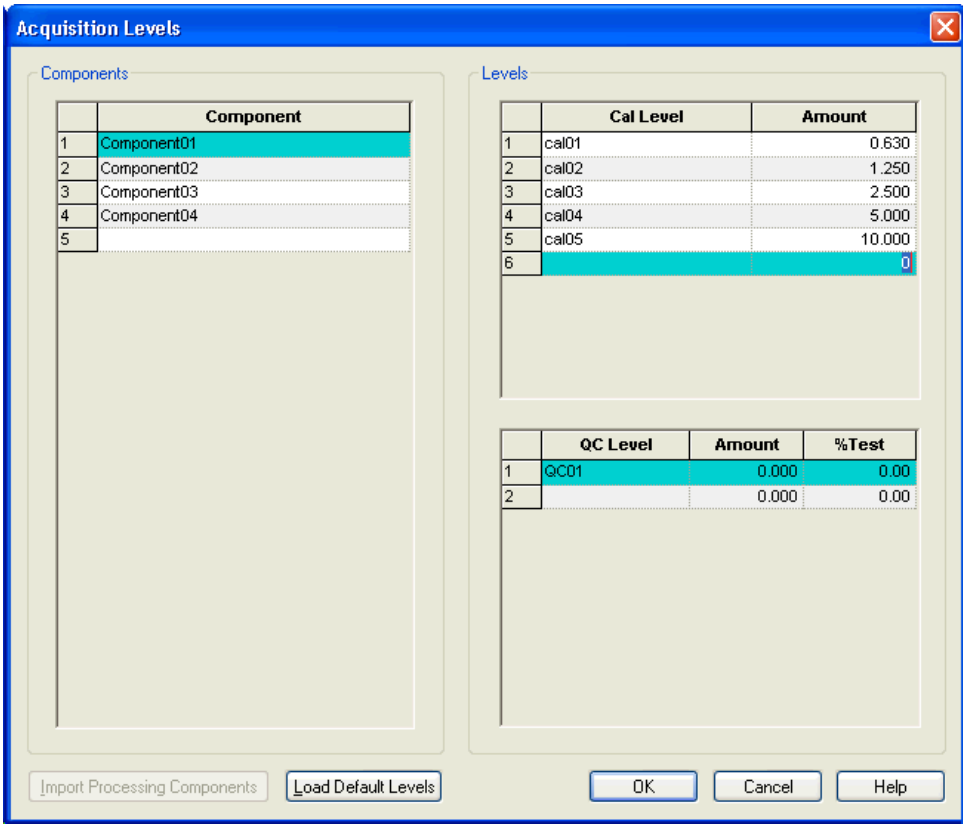
Right-click in the Sequence table and choose **User Labels and Values** from the shortcut menu to display the Change Sequence User Labels dialog box. In the User Label text boxes, type the label for each entry. In the User Values text boxes, either type the text to appear in each box or select a macro name from the drop down list. When you select a macro name LCquan displays the actual text that corresponds to the macro name in the Sequence Header. For example, if you select *$Workbook*, LCquan displays the name of the current workbook in the field in the Sequence Header.

Any changes that you make to the user labels or values with the Change Sequence User Labels dialog box are applied to all rows in the Acquisition Sequence. Any new rows that you create use the same labels and values.

Assigning Calibration or QC Levels

You can define or modify the calibration levels and QC levels that are specified in the Acquisition Sequence by using the Acquisition Levels dialog box. To open this dialog box, right-click in the Sequence table and choose **Standard and QC Levels** from the shortcut menu. See Figure 20.

Figure 19. Acquisition Levels dialog box



The Acquisition Levels dialog box contains the Components table and the Levels tables. You can enter the names of the components present in your sample in the Component table and then define calibration or QC levels for each component. If you have already defined components in the Quantitate view, you can import them into the Processing table by clicking the **Import Processing Components** button.

Any calibration or QC levels that you entered into the Acquisition Sequence appear in the Levels tables. You can define the amount for each calibration level and the amount and %test (the acceptable difference between the known and measured amount) for each QC level. You can also create and define new levels. If you want to load the set of default levels, click on the **Load Default Levels** button.

When you select a component in the Component table, the shortcut menu contains the command **Copy Current Component Levels to All Target Compounds** to copy the level settings for the selected component to all other components or **Load Default Levels for Current Component** to load default levels for the selected component. To access the shortcut menu, right-click anywhere in the dialog box.

After you define components and levels in the Acquisition Levels dialog box, this information can be transferred into the Quantitate view to use when you create your Processing Method and Processing Sequence.

Printing and Exporting the Acquisition Sequence

You can print the Acquisition Sequence information in several formats: You can print a brief listing of the samples arranged in order of the autosampler vial position. You can print the entire sequence with all of the columns. Or, you can print the sequence with only the columns that are displayed in the Sequence table. To print, choose **File > Print Sequence Info**. The Print Selection dialog box opens and allows you to select the format of the printing output.

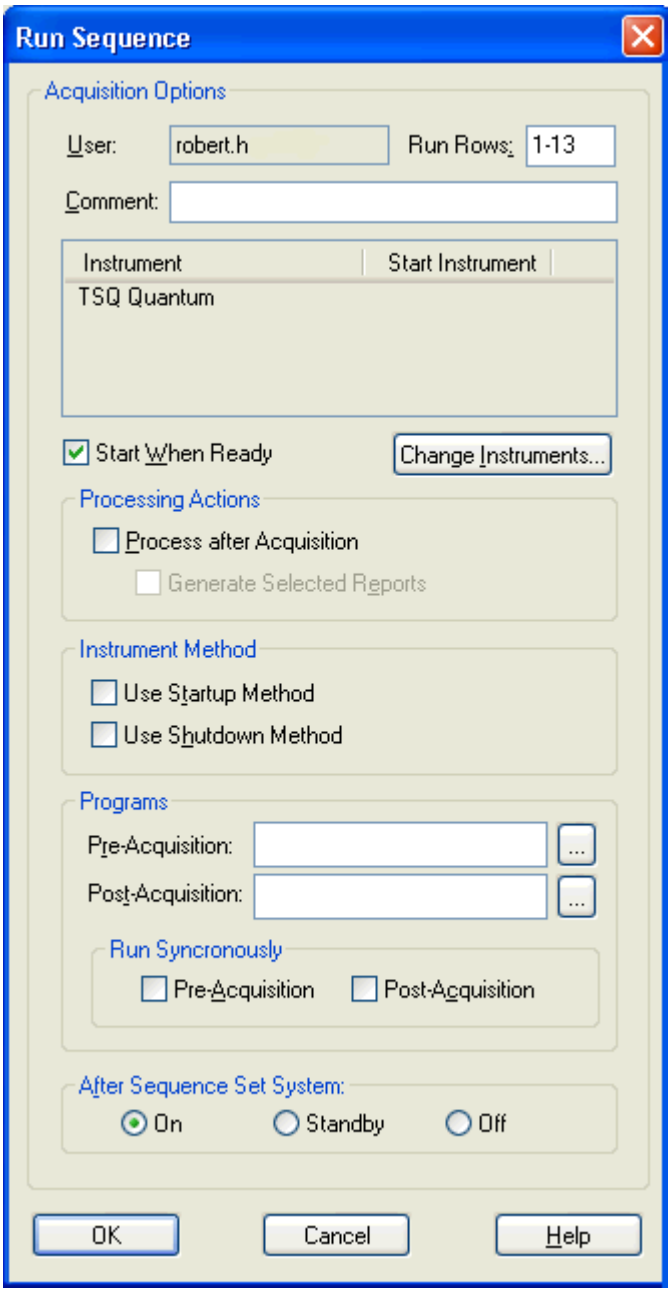
In addition, you can export the Acquisition Sequence. To export, choose **File > Export Acquisition Sequence**. The Save As dialog box opens and allows you to save the sequence file (*.sld*). When you export a file, LCquan places a copy of the file in the *Exports* folder for the workbook.

Run Sequence Dialog Box

The Acquisition view – Run Sequence dialog box allows you to select acquisition options and processing actions for the Acquisition Sequence. It also allows you to run one sample or a sequence of samples.

After you have created your Acquisition Sequence, click on the **Acquire** button in the viewbar to open the Acquisition view – Run Sequence dialog box. See Figure 20.

Figure 20. Acquisition view – Run Sequence dialog box



❖ To run a series of samples in the current Acquisition Sequence

1. In the Run Rows text box, type the row numbers of the samples that you want to run. The rows must be consecutive.
2. In the Comment text box, type an optional comment to add to the Acquisition Sequence.
3. Specify the instruments that you want to run your samples:
   a. Click on **Change Instruments** to open the Change Instruments In Use dialog box.
   b. Specify the instruments that you want to use and the start instrument for the current Acquisition Sequence by clicking in the In Use and Start Instrument columns. *Yes* appears in the column to indicate that the instrument is selected.
   c. Click on **OK** to save changes and close the dialog box.
4. Specify whether or not to start the acquisition when the instruments are ready:
   - If you want LCquan to start the data acquisition when the instruments are ready, select the **Start When Ready** check box.
   - If you want LCquan to place the Acquisition Sequence in the paused state after all of the devices are ready, **clear** the **Start When Ready** check box. You must manually start the acquisition by using the control buttons in the Acquisition view – Status page.
5. Specify the processing actions:

   Note LCquan can automatically process the data after it is acquired. However, a Processing Method must already be defined and the acquisition component names must match the processing component names.

   - If you want LCquan to process the data after it is acquired, select the Process After Acquisition check box. Then, if you want to have LCquan print Sample Reports and Summary Reports after the data is processed, select the **Generate Selected Reports** check box.
   - If you do not want LCquan to process the data after it is acquired, **clear** the **Process After Acquisition** check box.
6. Specify any optional Startup or Shutdown Instrument Methods:
   - If you want the Startup Method to be run before the sequence is run, select the **Use Startup Method** check box.
   - If you want the Shutdown Method to be run after the sequence is run, select the **Use Shutdown Method** check box.
   - If you do not want these methods run, clear the check boxes.

7. Specify any optional pre-acquisition or post-acquisition programs:
   a. In the Pre-Acquisition text box, specify the pre-acquisition program. You can use the Browse button to locate the program.
   b. In the Post-Acquisition text box, specify the post-acquisition program. You can use the Browse button to locate the program.
   c. Specify whether to run these programs synchronously (in series) or asynchronously (in parallel) with the data acquisition:
      - If you want to run these programs synchronously, select the **Run Synchronously: Pre-Acquisition** check box and the **Run Synchronously: Post-Acquisition** check box.
      - If you want to run these programs asynchronously, **clear** the **Run Synchronously: Pre-Acquisition** check box and the **Run Synchronously: Post-Acquisition** check box.
8. Specify the status of the system after data acquisition by selecting one of the After Sequence Set System option buttons: **On**, **Standby**, or **Off**.
9. Click on **OK** to save the settings and close the dialog box. LCquan places the selected samples at the end of the Acquisition Queue and opens the Acquisition view – Status page.

For more information, see the online Help available within the software.

Note If you acquire data, LCquan requires that you save the workbook.

Acquisition View – Status Page

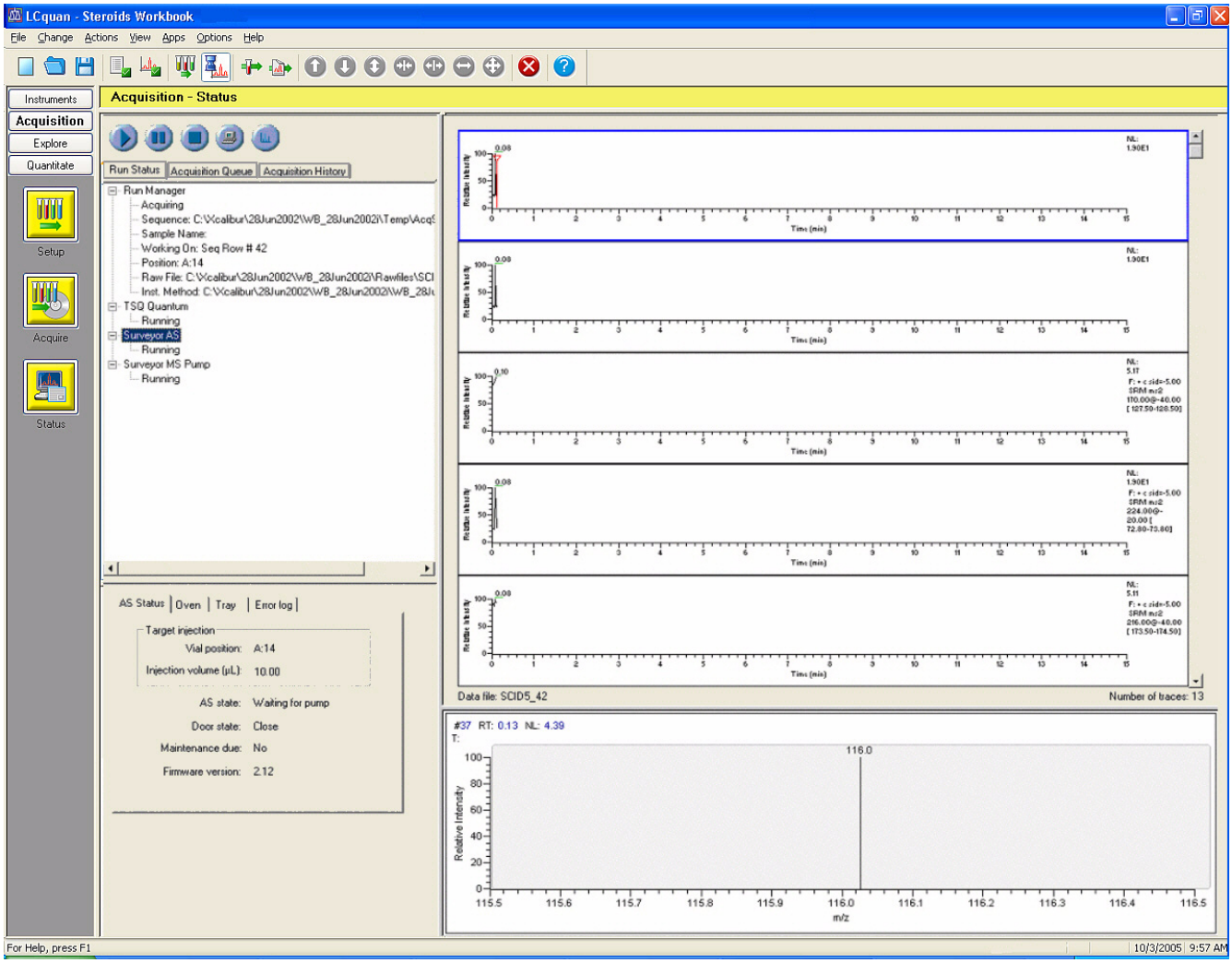
The Acquisition view – Status page provides a real-time display of the data acquisition. The following subtopics provide information on this page:

- Overview
- Using the Chromatogram Pane
- Using the Spectrum Pane

Overview

The Acquisition view – Status page consists of a set of control buttons, an information pane that contains the Run Manager Status and the Instrument Status, the Acquisition Queue, and the Acquisition Sequence History; the Chromatogram pane; and the Spectrum pane. See Figure 21.

Figure 21. Acquisition view – Status page



The ***control buttons*** allow you to modify the acquisition or real-time display. They are described in Table 3.

Table 3. Control buttons in the Acquisition view – Status page and their functions

Control Button	Function
Start/Resume	Starts an acquisition or restarts an acquisition that has been paused.
Pause	Pauses the acquisition after the current sample has been completely acquired. When clicked on again, it restarts the acquisition.
Stop	Allows you to stop the acquisition of a sample or sequence that belongs to the current workbook. You can choose to stop the acquisition of the current sample and pause, to stop the acquisition of the current sequence and pause, or to stop the acquisition of all sequences.
Screen Pause	Pauses the chromatogram and spectrum display. The acquisition continues. When clicked on again, it restarts the display.
Display Spectrum	Displays the Spectrum pane below the Chromatogram pane. When clicked on again, it hides the Spectrum pane.

The ***Run Manager Status*** provides a summary of the acquisition progress. It lists the sequence and sample information and the readback status of each configured instrument.

The ***Instrument Status*** provides the complete readback status of each configured instrument.

The ***Acquisition Queue*** displays all of the sequences that have been submitted for acquisition, including those for other workbooks. The sequences and their individual sequence rows are organized in a tree view control. Each row of the tree view control contains a check box to indicate when it is selected or not selected. While a sample is being acquired, its icon has a green background. After the sample has been acquired, the background changes to blue and a circle with an X through it appears in the check box.

You can remove a sequence or sequence row that has not been acquired from the Acquisition Queue by first selecting it and then pressing DELETE. (However, you cannot delete a sequence that another workbook or application submitted.) You can view specific information about an individual sample by right-clicking on the row in the queue and selecting **Properties** from the shortcut menu. To display the Acquisition Queue, click on the Acquisition Queue tab.

The ***Acquisition Sequence History*** lists every Acquisition Sequence that was submitted to the acquisition queue in the life of the workbook. It provides information on each sequence, including the operator, the start and end times, comments, and the status of the sequence, as well as the calibration and QC level data. While LCquan is acquiring data, the history is dynamically updated with samples that have been fully acquired. To display the history, click on the Acquisition History tab.

The ***Chromatogram pane*** of the Acquisition Status view allows you to monitor chromatograms for each of the defined components plus up to ten user defined chromatograms in real time (up to five are visible at one time). It can also display data from an existing raw file.

The ***Spectrum pane*** allows you to monitor the mass spectrum in real time or to view the mass spectrum of a selected raw file.

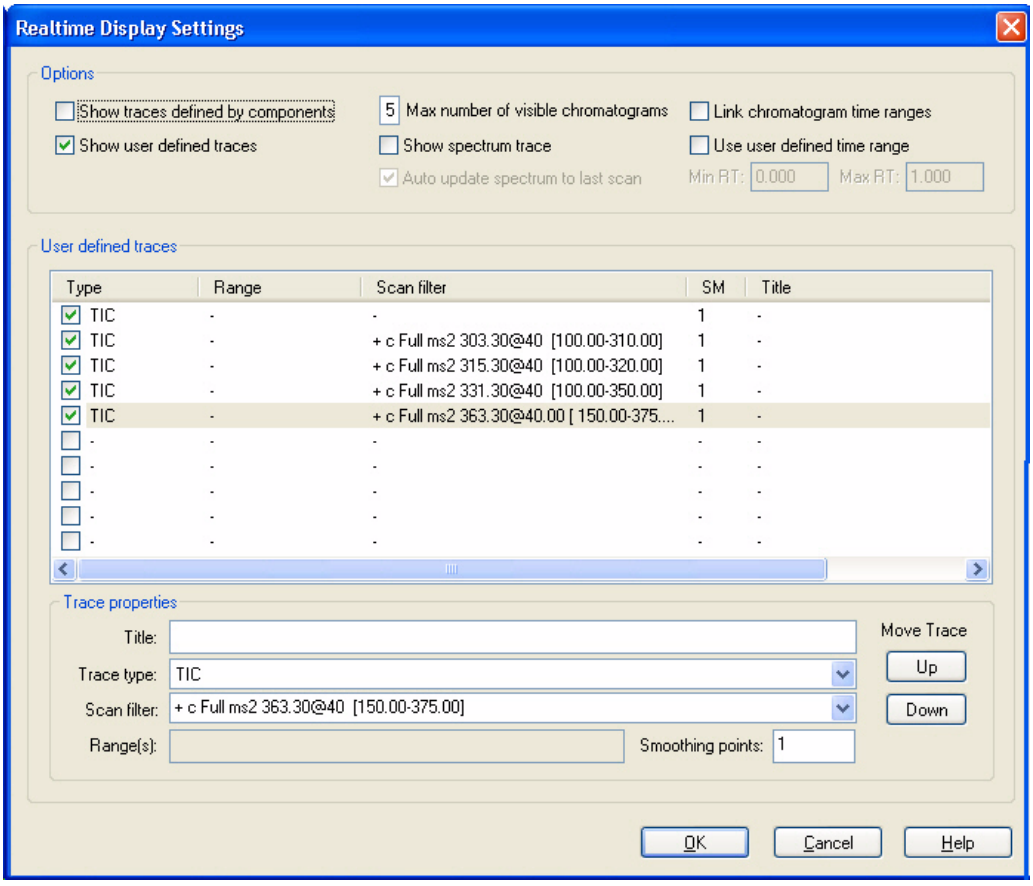
Using the Chromatogram Pane

You specify the properties of the chromatograms that LCquan displays by using the Realtime Display Settings dialog box. To open the Realtime Display Settings dialog box, choose **Options > Realtime Display Settings**. See Figure 22. This dialog box consists of two parts: the Options group box and the User Defined traces group box. The settings in the Options group box allow you to specify the types and number of chromatograms to display, the time range information for the chromatograms, and whether or not to display the spectrum trace in the Spectrum pane. (Many of these options also appear in a shortcut menu that can be accessed by right-clicking in the Chromatogram pane.) The elements of the User Defined Traces group box allow you to specify the settings for up to ten chromatograms. For more detailed information about using the Realtime Display Settings dialog box, refer to the online Help.

While LCquan is acquiring data, you can also display an existing raw file in the Chromatogram pane using the current set of filters and settings. LCquan continues to acquire data while displaying a raw file, but the screen is in pause mode. To display a raw file, right-click in the Chromatogram pane and choose **Display Raw File** from the shortcut menu. The Select Rawfile dialog box appears from which you can choose the appropriate raw file. You can also display a raw file by dragging it from the WindoW® Explorer and dropping it into the Chromatogram pane or by dragging an older sample from the Acquisition Sequence History and dropping it into the Chromatogram pane.

With any chromatogram, you can use the display buttons in the toolbar or the items in the Zoom menu to zoom in or out along the axes. You can also click and drag with the mouse in the Chromatogram pane to zoom in. When you zoom in, the trace does not expand if the data extends out of the pane.

Figure 22. Realtime Display Settings dialog box, showing the settings for five user-defined traces



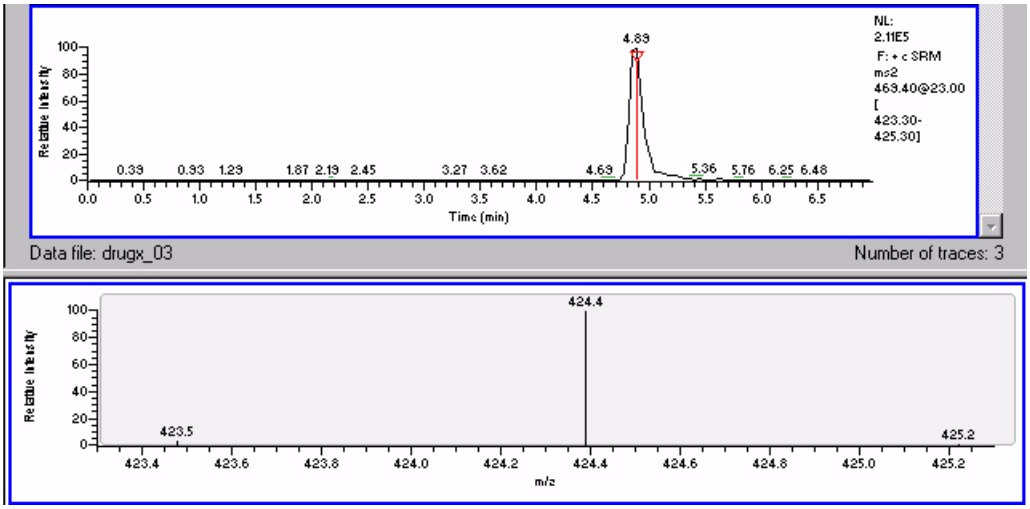
Using the Spectrum Pane

The Spectrum pane displays the data for the currently selected scan in the currently selected chromatogram. Select a scan by left-clicking on a chromatogram. A red vertical line appears at the scan you selected in the Chromatogram pane and the spectrum that corresponds to that scan appears in the Spectrum pane. See Figure 23.

You can choose to apply a scan filter to the data based on the selected chromatogram. Right-click in the Spectrum pane and choose **Filter Spectrum** from the shortcut menu. A check mark appears next to the command. To remove the filter and show every scan, choose this command again.

You can use the display buttons in the toolbar or the commands in the Zoom menu to zoom in or out along the axes of the spectrum. You can also click and drag with the mouse in the Spectrum pane to zoom in.

Figure 23. Chromatogram pane and Spectrum pane, showing a selected scan



Processing Data While Continuing to Acquire Data

While you are acquiring data you can enter the Quantitate view to process the data already acquired. If you have not yet defined a Processing Method or a Processing Sequence, you need to complete these steps first. Refer to Chapter 4, "Creating a Processing Method." and Chapter 5, "Creating a Processing Sequence."

To enter the Quantitate view, click on the **Quantitate** Quantitate button in the viewbar. You can choose to use the defined Processing Sequence or you can replace the defined Processing Sequence with a shortened sequence that is based on the data already acquired.

You can also specify whether the current Processing Sequence is updated with the most recent list of samples acquired. From either the Acquisition view or the Quantitate view, choose **Options > Acquisition Sequence Info** to open the Acquisition Sequence dialog box. Select the **Auto Update the Processing Sequence When Acquiring** check box to update the sequence. If you are in the Quantitate view and currently acquiring data when you select this option, a message box prompts you to update the current Processing Sequence with the most recent list of samples acquired. If you do not respond to the message box after 5 s, LCquan automatically updates the sequence.

Exploring Your Data

This chapter describes the features of the Explore view. You can use the Explore view to display a multi-component chromatogram, and then to experiment with peak detection and integration parameters to see how they affect the chromatogram.

Contents

- About the Explore View
- Opening a Raw File and Displaying the Chromatogram
- Generating a Peak List
- Identifying Components
- Reviewing the Explore Results
- Creating a Quan Component from the Peak List
- Additional Features of the Review and Explore Results Page

About the Explore View

The Explore view allows you to display a multi-component chromatogram, and then to experiment with peak detection and integration parameters to see how they affect the chromatogram. In the Explore view, you can also create and export quan components to the Processing Method to get it started. In Explore it's expected that you'll be looking at the entire chromatogram and find multiple peaks.

The Explore view of LCquan is similar to the Qual view of Processing Setup in Xcalibur. Some features of the Explore view are:

- You can generate quan components automatically by using the peak list function.
- Explore view displays the Acquisition Sequence History and allows you to select a sample from it.
- Explore view does not allow you to edit sequences, but you can import several sequences to review, and select samples from any of the imported sequences.
- Things you do in the Explore view are not part of the audit trail (except for exporting quan components to the Quantitate view).

Opening a Raw File and Displaying the Chromatogram

You open a raw file that is representative of your data set to determine suitable peak detection and integration parameters. In this tutorial, you use the *steroids04.raw* file. In general, you choose a raw file corresponding to a low-concentration calibration standard.

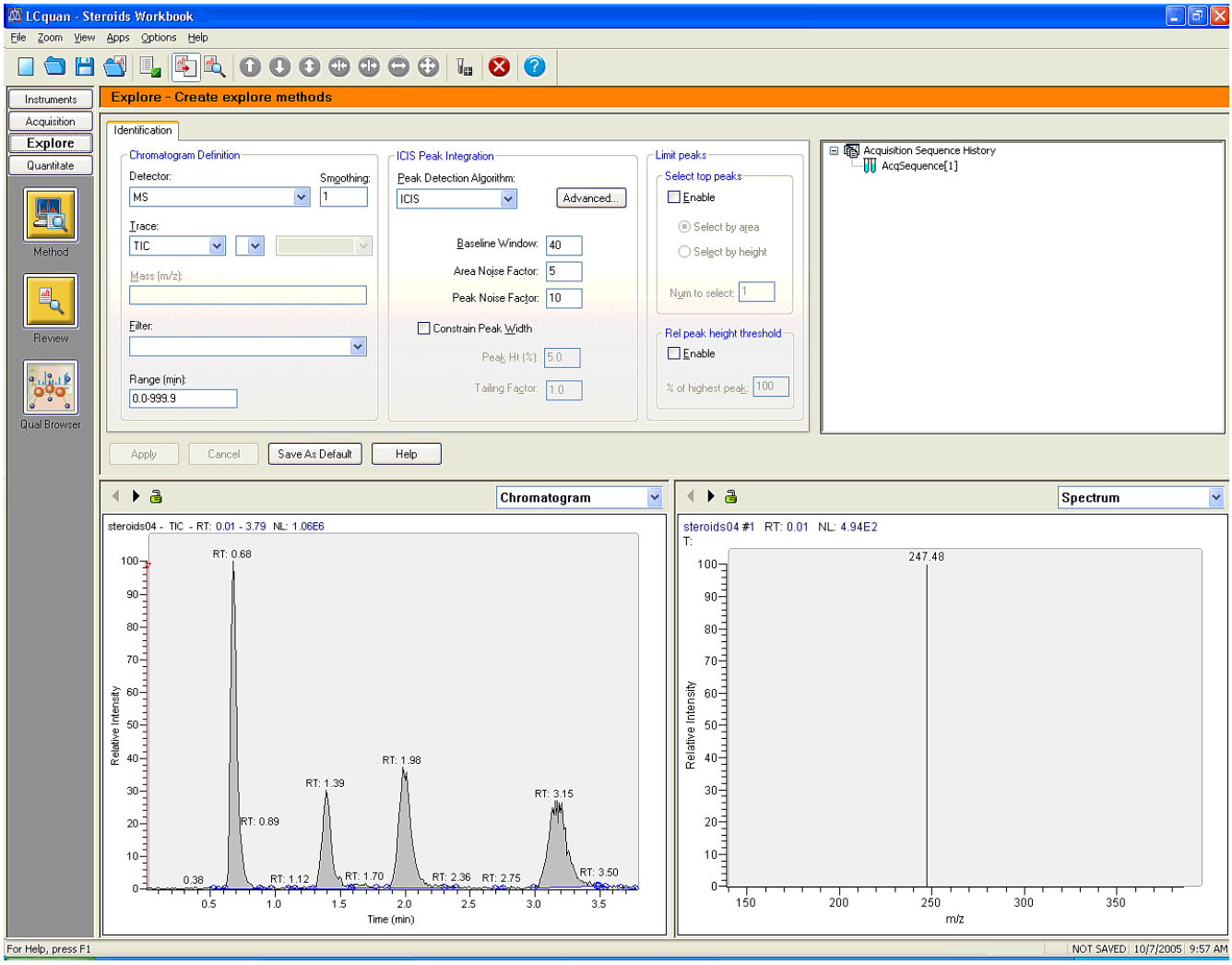
❖ To enter the Explore view and open the *steroids04* raw file

1. Click on the **Explore** button to enter the Explore view. LCquan displays the Explore – Create Explore Methods page.
2. Open the *steroid04* raw file:
   a. Choose **File > Open Raw File** to open the Select Raw File dialog box.
   b. Browse to the *Rawfiles* folder or the *Xcalibur\Examples\Data* folder.
   c. Select ***steroids04.raw*** and click **Open**.

   LCquan imports the raw data for the *steroids04.raw* sample and displays the raw chromatogram in the Create Explore Methods page of the Explore view. See Figure 24.

Four peaks, which correspond to the four steroids, appear in the chromatogram at retention times of 0.68, 1.39, 1.98, and 3.15 min.

Figure 24. Explore view – Create Explore Methods page, showing the raw chromatogram for steroids04 (Note that you must click on a peak in the chromatogram to see a proper mass spectrum.)



Generating a Peak List

LCquan can generate a peak list. A ***peak list*** is a table of peak properties such as retention times and peak areas of the major peaks in the chromatogram. You can use the peak list to identify and name quan components, and then export the component names and properties to the Quantitate view. See the topic: "Creating a Quan Component from the Peak List" on page 61.

❖ To create a peak list corresponding to the four major peaks of the steroids example

1. Select **Peak List** in the right-side pane.
2. Specify the peak list parameters:
   - Select Top Peaks: Enabled:
   - Select by Area:
   - Num to Select: **4**
3. Click on **Apply**.
4. LCquan generates a peak list for the four major peaks in the chromatogram. See Figure 25.

> **Note** In Explore, only the peak name is editable in the peak list.

You can have LCquan generate names for the peaks by right-clicking on the peak list and then choosing **Create Peak Names > For All Peaks in List** from the shortcut menu. LCquan generates peak names in the form "Peak @ x.xxxx" where x.xxxx is the retention time at the peak apex. (The component name is the same as the peak name.) See Figure 26.

Figure 25. Explore view – Create Explore Methods page, showing the peak list for the steroids example

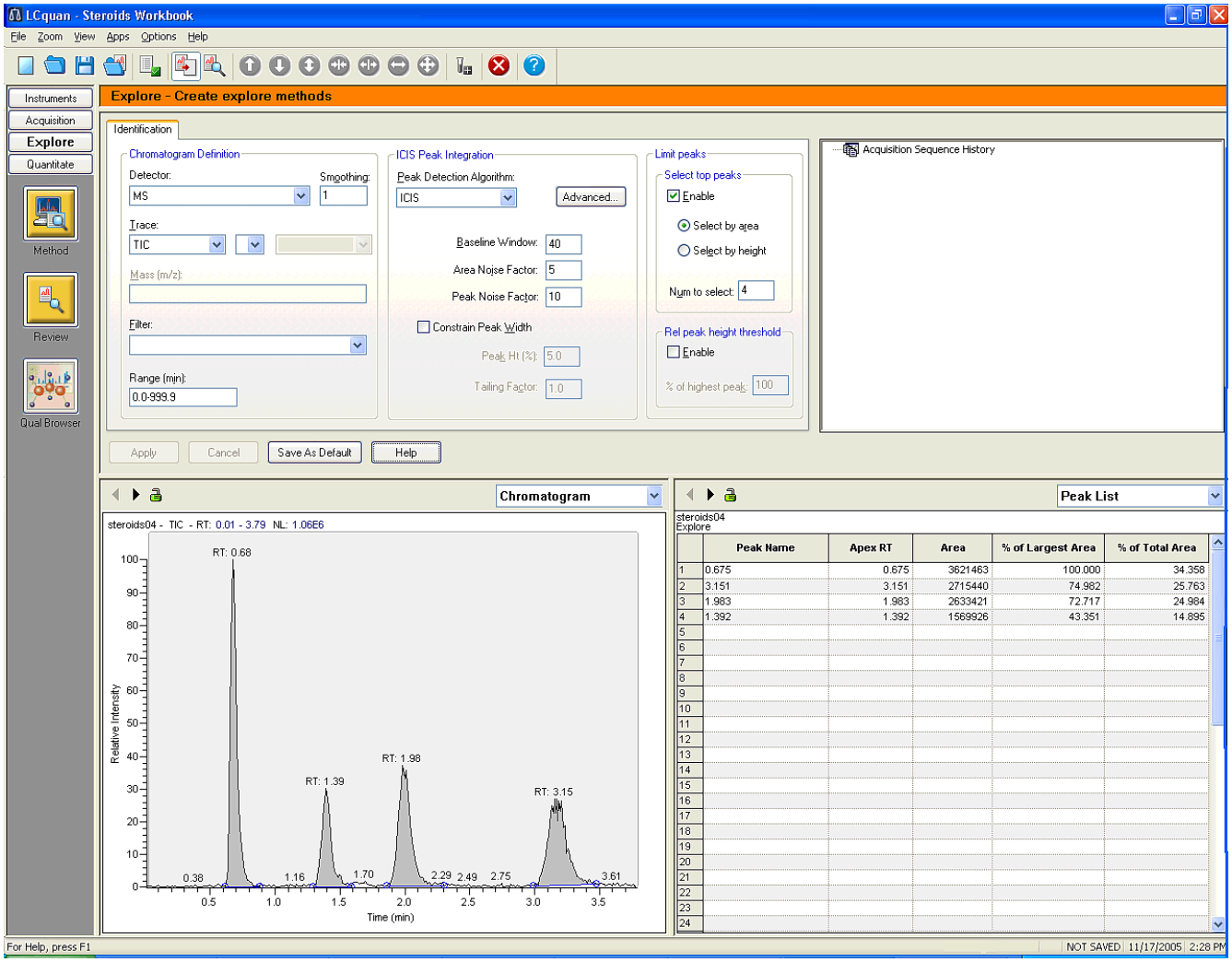
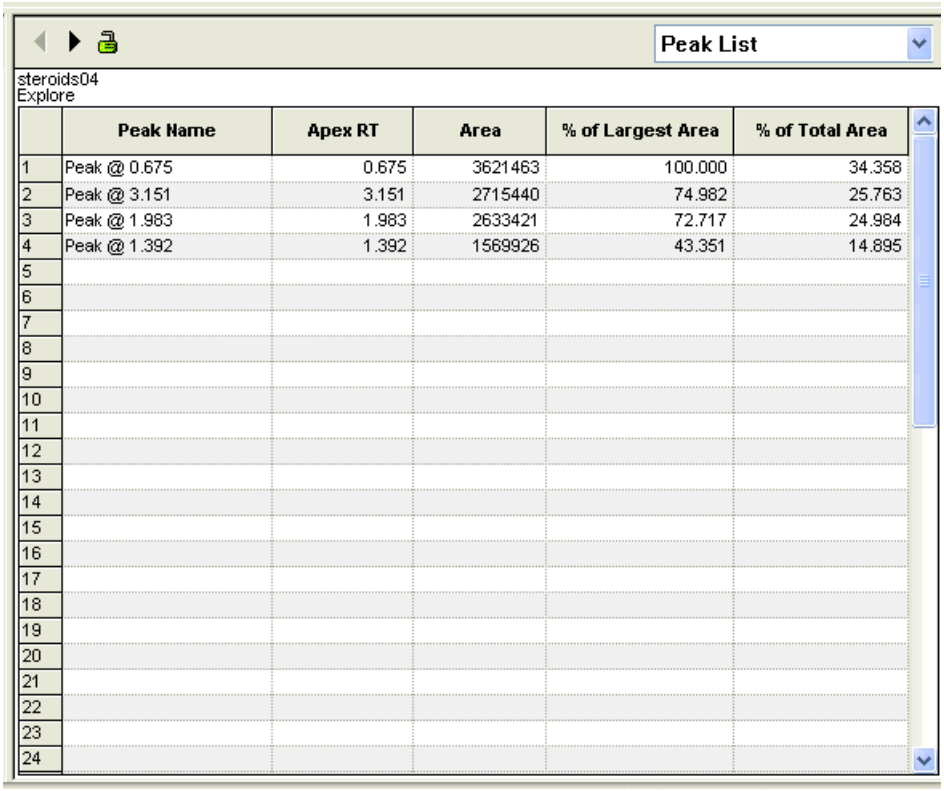


Figure 26. Peak list with auto-generated names



	Peak Name	Apex RT	Area	% of Largest Area	% of Total Area
1	Peak @ 0.675	0.675	3621463	100.000	34.358
2	Peak @ 3.151	3.151	2715440	74.982	25.763
3	Peak @ 1.983	1.983	2633421	72.717	24.984
4	Peak @ 1.392	1.392	1569926	43.351	14.895
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					

You can change peak names by selecting the current name and then typing a new name, or by using the Peak Names List Editor. To open the Peak Names List Editor, right-click on the peak list and choose **Modify Peak Names** from the shortcut menu. The Peak Names List Editor also allows you to import and export peak lists.

Once you name the components, you can export the component names and settings to the Quantitate view by right-clicking on the peak list and then choosing **Create Quan Components > For All Peaks in List** from the shortcut menu. See the topic: "Creating a Quan Component from the Peak List" on page 61.

Go on to the next topic "Identifying Components".

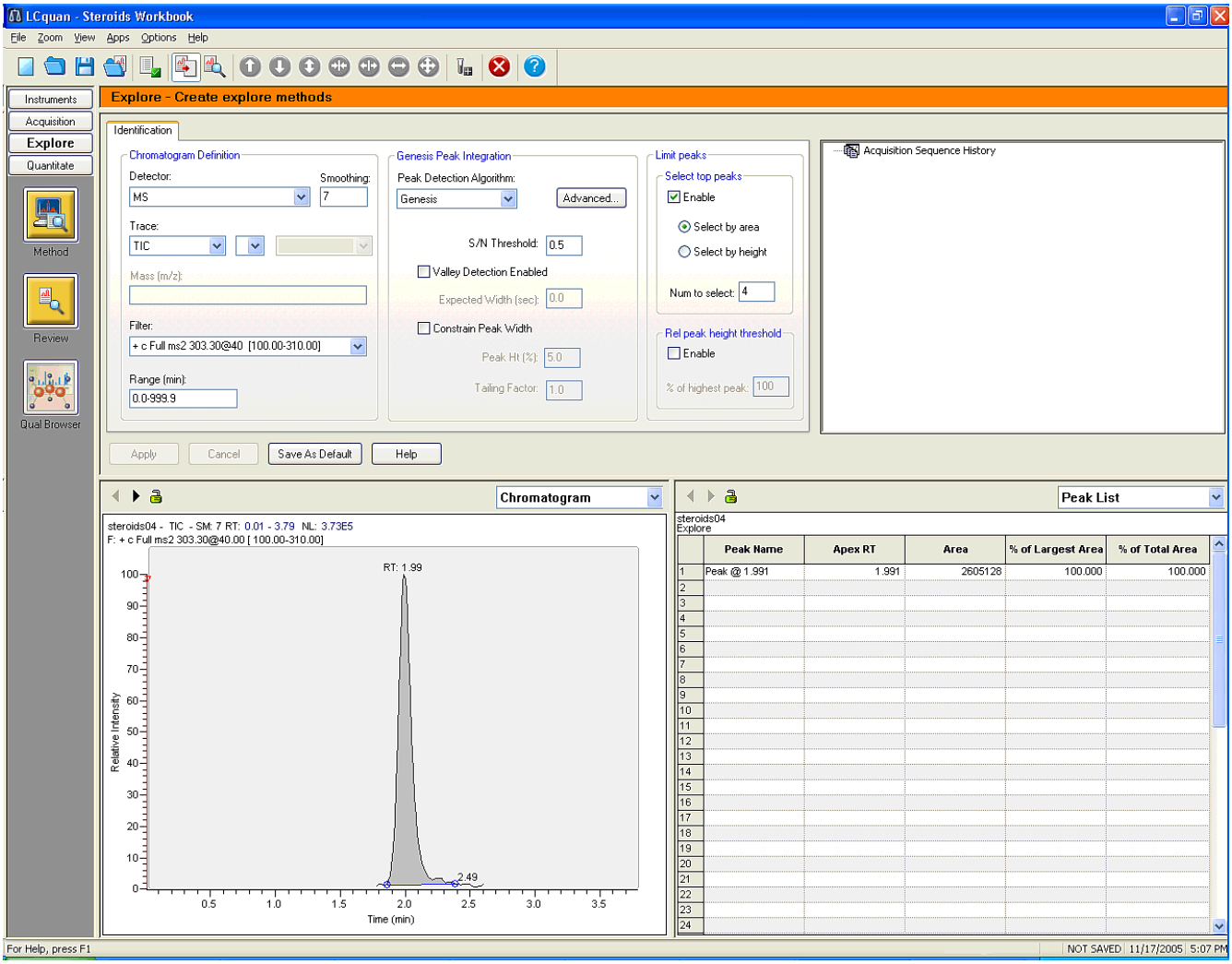
Identifying Components

When LCquan acquires data, it creates unique scan filters according to the type of experiment you specify in the Instrument Method. When you load a raw file, LCquan lists in the Filter box the scan filters associated with the raw file. In this example, full scan MS/MS data were acquired on the steroids by using alternating product ion scans.

❖ To relate the chromatogram peaks with the steroids

1. Select the scan filter for methyltestosterone (parent ion mass *m/z* 303), Filter: **+ c Full ms2 303.30@40 [100.00-310.00]**, and click **Apply**. LCquan displays the filtered chromatogram. See Figure 27. Note that methyltestosterone corresponds to the peak named *Peak @ 1.991* in the peak list.

Figure 27. Explore view – Create Explore Methods page, showing a filtered chromatogram for methyltestosterone



2. Select the scan filter for deoxycorticosterone (parent ion mass *m/z* 331), Filter: **+ c Full ms2 331.30@40 [100.00-350.00]**, and click **Apply** to display the filtered chromatogram. Note that deoxycorticosterone corresponds to the peak named *Peak @ 1.392* in the peak list.

3. Select the scan filter for hydrocortisone (parent ion mass *m/z* 363), Filter: **+ c Full ms2 363.30@40 [150.00-375.00]**, and click **Apply** to display the filtered chromatogram. Note that hydrocortisone corresponds to the peak named *Peak @ 0.675* in the peak list.

4. Select the scan filter for progesterone (parent ion mass *m/z* 315), Filter: **+ c Full ms2 315.30@40 [100.00-320.00]**, and click **Apply** to display the filtered chromatogram. Note that progesterone corresponds to the peak named *Peak @ 3.151* in the peak list.

5. Select Smoothing: **7**and Peak Detection Algorithm: **Genesis** and click **Apply**. Notice the effect on the progesterone peak.

   Note The Explore view is where you experiment with the peak detection and peak integration parameters. Refer to the Help for descriptions of the peak detection and peak integration parameters.

6. **Clear** the Filter box, click **Apply**.

Go on to the next topic: "Reviewing the Explore Results".

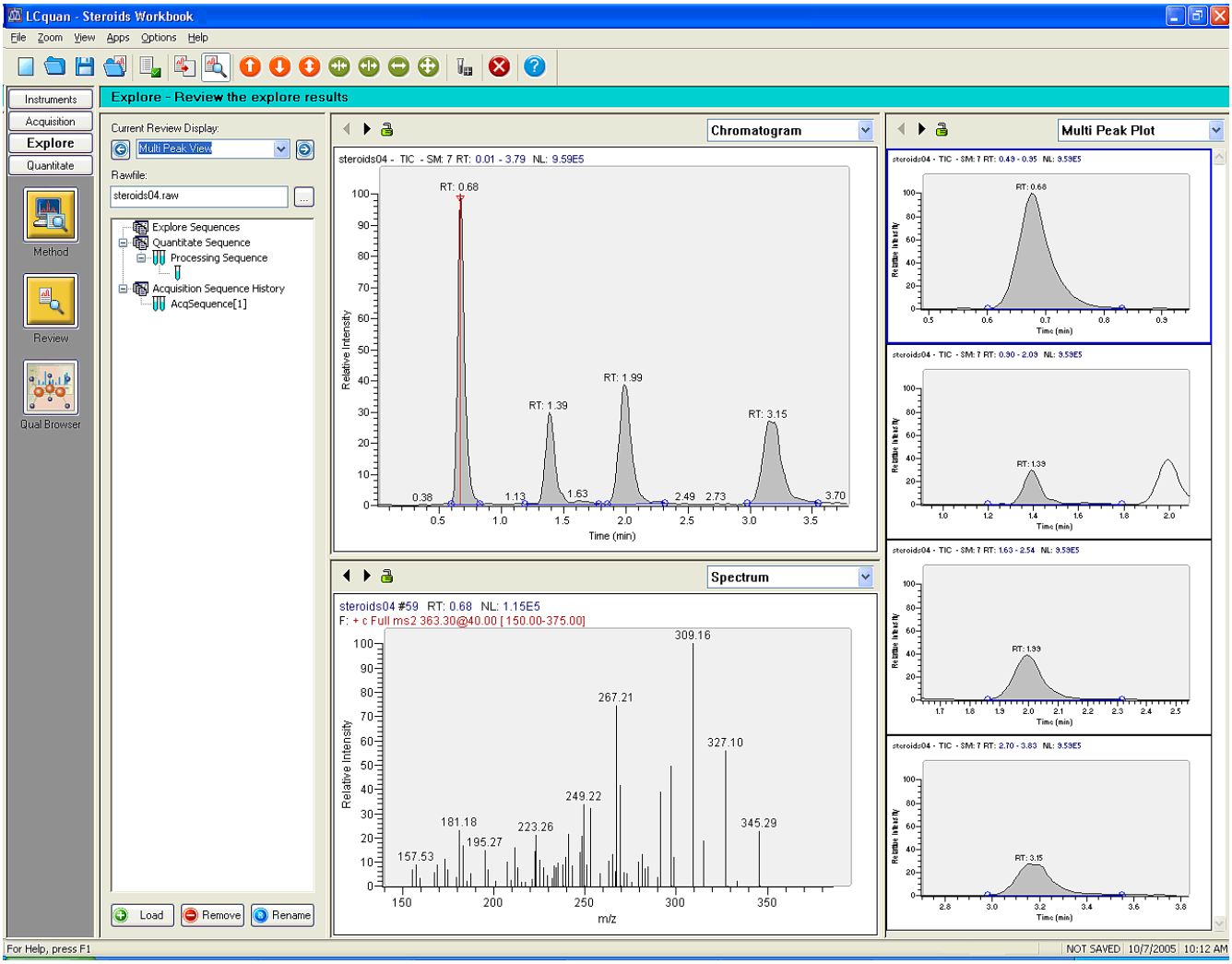
Reviewing the Explore Results

In this section you review the filtered chromatograms and mass spectra of the steroids. The unfiltered chromatogram is shown in Figure 28.

❖ To review the Explore results

1. Click on the **Review** button. LCquan displays the Review the Explore Results page. See Figure 28.
2. Select **Multi Peak Plot** in the right-side pane.
3. Click on the top chromatogram peak (hydrocortisone) in the multipeak plot. LCquan displays the product ion mass spectrum at the maximum intensity.
4. Record the mass-to-charge ratios of the first several ions of highest intensity in the hydrocortisone mass spectrum.

Figure 28. Explore view – Review the Explore Results page, showing the unfiltered chromatogram for the steroids



5. Click on the second chromatogram peak (deoxycorticosterone) in the multipeak plot. LCquan displays the product ion mass spectrum at the maximum intensity.
6. Record the mass-to-charge ratios of the first several ions of highest intensity in the deoxycorticosterone mass spectrum.
7. Click on the third chromatogram peak (methyltestosterone) in the multipeak plot. LCquan displays the product ion mass spectrum at the maximum intensity.
8. Record the mass-to-charge ratios of the first several ions of highest intensity in the methyltestosterone mass spectrum.
9. Click on the fourth chromatogram peak (progesterone) in the multipeak plot. LCquan displays the product ion mass spectrum at the maximum intensity.
10. Record the mass-to-charge ratios of the first several ions of highest intensity in the progesterone mass spectrum.

The three most intense product ions for the steroids are listed in Table 4.

Table 4. Mass-to-charge ratios of the three most intense product ions of the steroids mass spectra

hydrocortisone	deoxycorticosterone	methyltestosterone	progesterone
309.2	253.1	285.1	297.1
267.2	295.2	267.3	279.2
327.1	109.0	227.2	255.0

You now have enough information to create quan components. Go on to the next topic: "Creating a Quan Component from the Peak List".

Creating a Quan Component from the Peak List

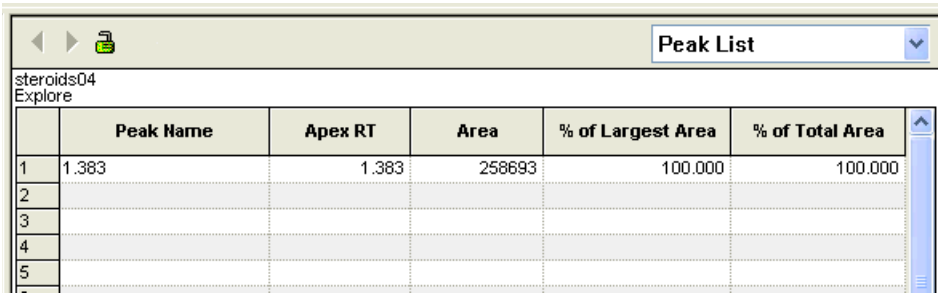
This topic demonstrates how to create a quan component in the Explore view, and then export it to the Quantitate view. Creating quan components is the first step in creating a Processing Method.

The components that LCquan creates out of the peak list are based on the Explore Method that you define in the Create Explore Methods page. In the previous topic, you set up a method that's a TIC (no filter). For steroids, this resulted in a chromatogram and peak list with four peaks. In general, you want to filter first to get one peak in the peak list and then generate its quan component. You then add quan components to the Processing Method one at a time.

❖ To create the deoxycorticosterone quan component

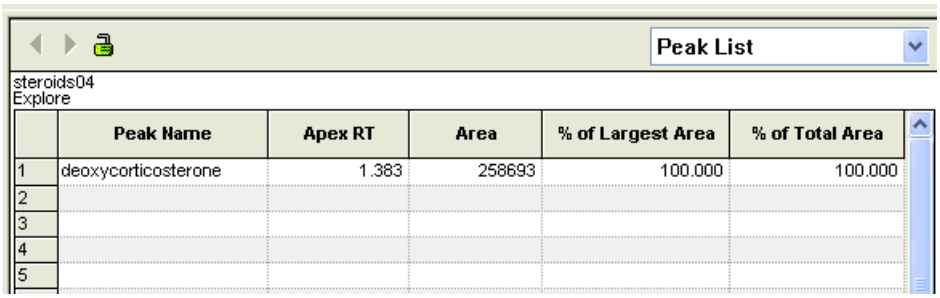
1. Click on the **Method** button to display the Create Explore Methods page.
2. Enter or select the following Explore Method settings:
   - Detector: **MS**
   - Trace: **Mass Range**
   - Masses: **109.0, 253.1, 295.2**
   - Smoothing: **7**
   - Peak Detection Algorithm: **Genesis**
   - Filter: **+ c Full ms2 331.30@40 [100.00-350.00]**
3. Change the bottom-right pane to **Peak List**.
4. Click on **Apply** to apply the settings. See Figure 29.
5. Select Peak Name: **1.383** and type in **deoxycorticosterone** to rename the peak. See Figure 30.

Figure 29. Initial peak list that was obtained with the Explore Method of step 2



	Peak Name	Apex RT	Area	% of Largest Area	% of Total Area
1	1.383	1.383	258693	100.000	100.000
2					
3					
4					
5					

Figure 30. Renamed peak list



6. Right-click on the peak list and select **Create Quan Components > From Selected Peaks in List** from the shortcut menu to export the deoxycorticosterone quan component to the Quantitate view – Create Method page.

For additional information on the Review and Explore Results page, go on to the next topic: "Additional Features of the Review and Explore Results Page".

To continue with the steroids example, go on to Chapter 4, "Creating a Processing Method."

Additional Features of the Review and Explore Results Page

Earlier in this chapter we described some useful features of the Explore view. For example, you can create an Explore Method, apply it to the raw data to create a peak list, and then used the peak list to create components for the Processing Method. The following are additional features of the Review and Explore Results page that are useful:

- Sample Selector
- Locks
- General Parameters Plot
- Status Plot
- Status Report
- Review Displays
- Exporting the Active Raw File to Qual Browser

Sample Selector

The ***sample selector*** allows you to easily select a raw file. See Figure 31. Click on a raw file in the sample selector to process that raw file against the Explore Method that you created in the Create Explore Methods page.

Three categories of sequences appear in the sample selector:

- Explore Sequence
- Quantitate Sequence
- Acquisition Sequence History

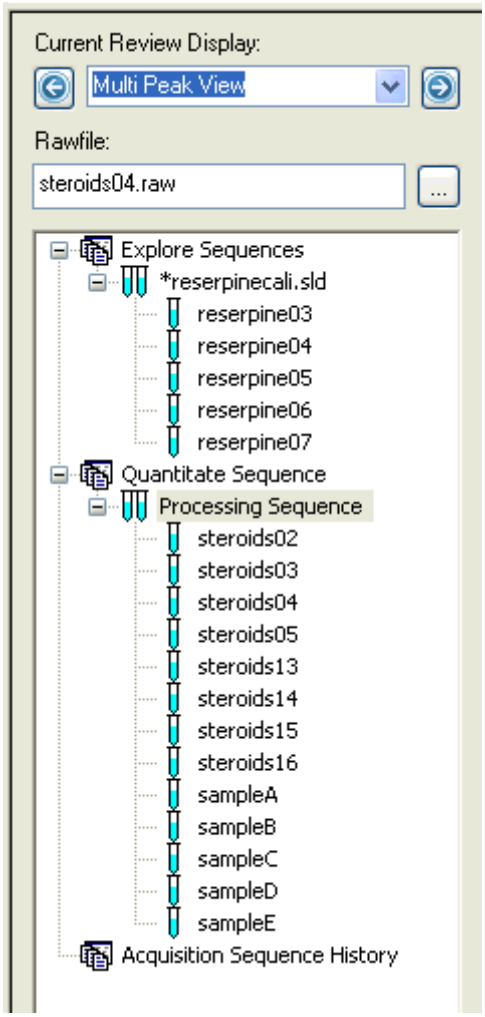
Explore Sequences are sequences that you imported into Explore by using the Load button. You can use the Remove or Rename button to delete or rename the selected sequence.

Quantitate Sequences are Processing Sequences that you previously created in the Quantitate view.

The ***Acquisition Sequence History*** is historical information about the sequences that were used to acquire the data in the current workbook.

Note Raw files use absolute paths. If you move the workbook, LCquan will not find the samples and will create an empty graph.

Figure 31. Sample selector



Locks

You use locks to compare data from different raw files simultaneously. For example, to see how the acquisition progressed from sample to sample. You can compare a plot from one sample to the plots of other samples by locking one plot and then clicking down the sequence in the sample selector.

You change the state of the lock by clicking on it. The lock can be in three different states:

- Unlocked (green)
- Locked against raw file change (yellow)
- Absolute lock (red)

When a plot is ***unlocked***, every change of raw file or parameter is reflected in the plot. Parameters, such as retention time, are sent out from unlocked plots, for example when you click on a chromatogram.

Plots that are ***locked against raw file change*** will respond to changes in parameters, but not to a change of raw file. Parameters are not sent out from plots that are locked against raw file change.

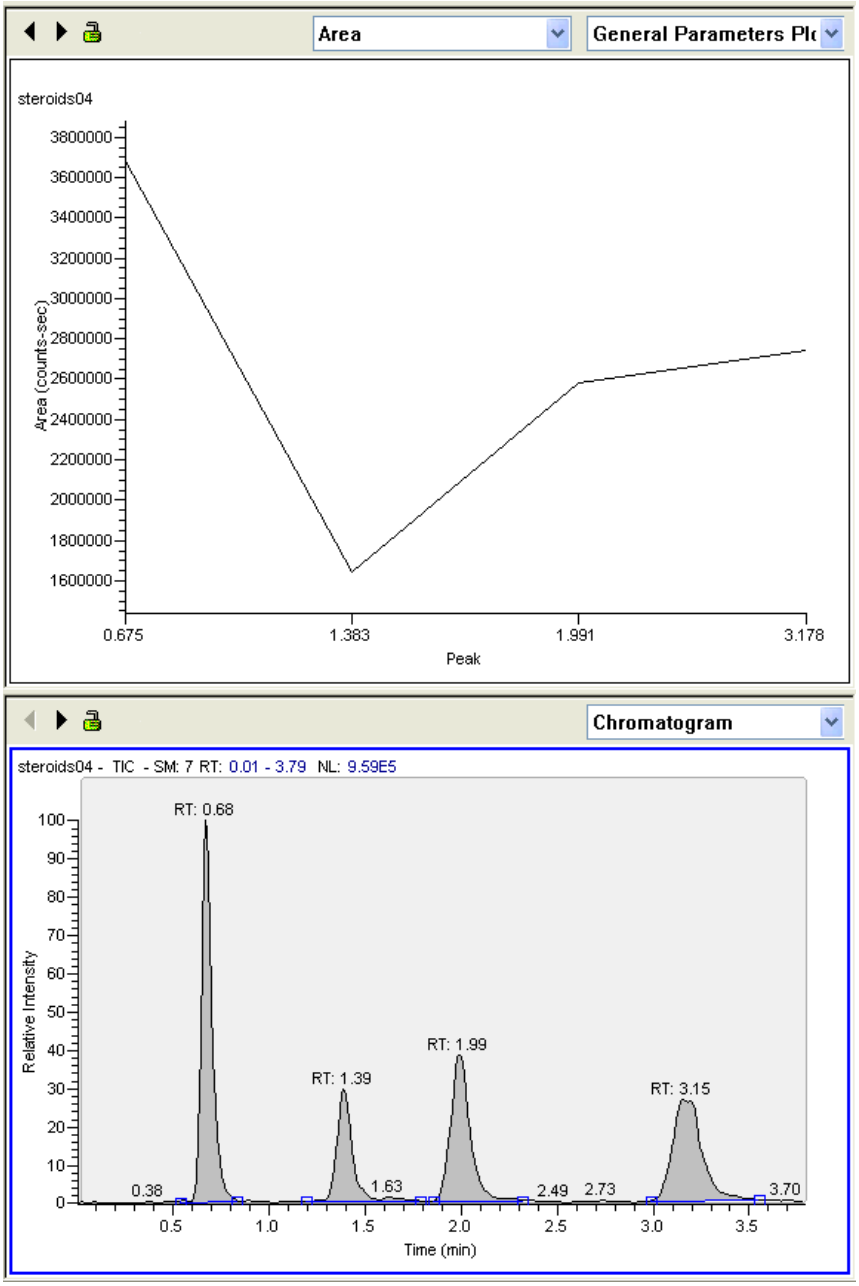
Plots that are ***absolutely locked*** will not respond to changes in parameters or raw files. Parameters are not sent out from plots that are absolutely locked.

General Parameters Plot

A ***general parameters plot*** provides a graphical representation of the retention time, peak area, peak height, or peak width across the chromatogram. A peak area general parameters plot for *steroids04* is shown in Figure 32.

You can lock the general parameters plot for one sample and click through the other samples to look for trends or inconsistencies in the data.

Figure 32. General parameter plot (top) and corresponding chromatogram (bottom) for steroids04

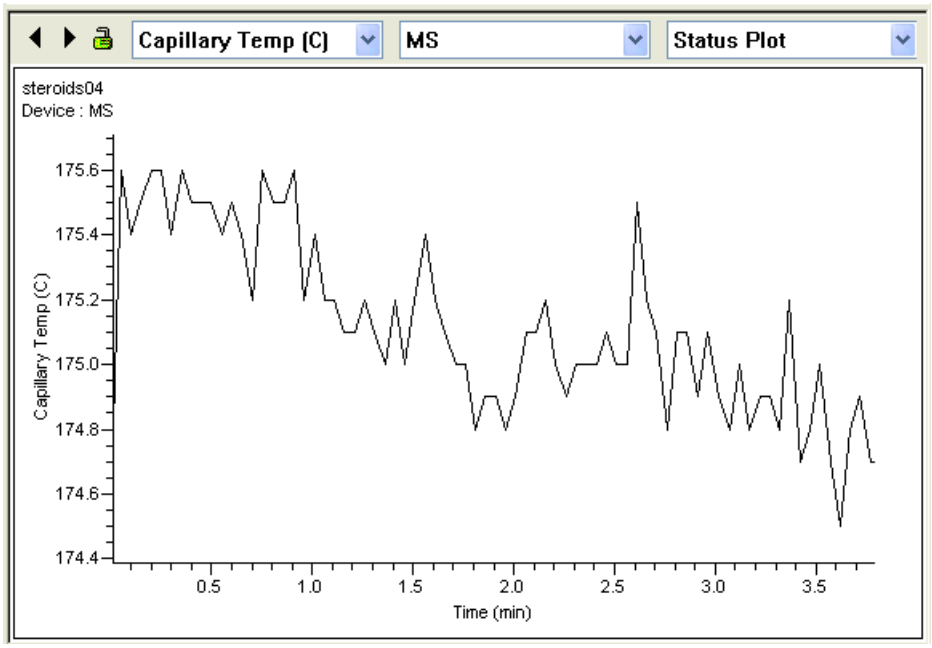


Status Plot

A ***status plot*** displays status data that is stored by instruments during the acquisition of the selected raw file. The drop down list lists all the parameters for the selected instrument. You can use the arrow buttons to move up or down the list.

A status plot of the capillary temperature is shown in Figure 33. Status plots can be used to investigate an anomaly in a chromatogram or to verify that the Instrument Method did what you expected it to do.

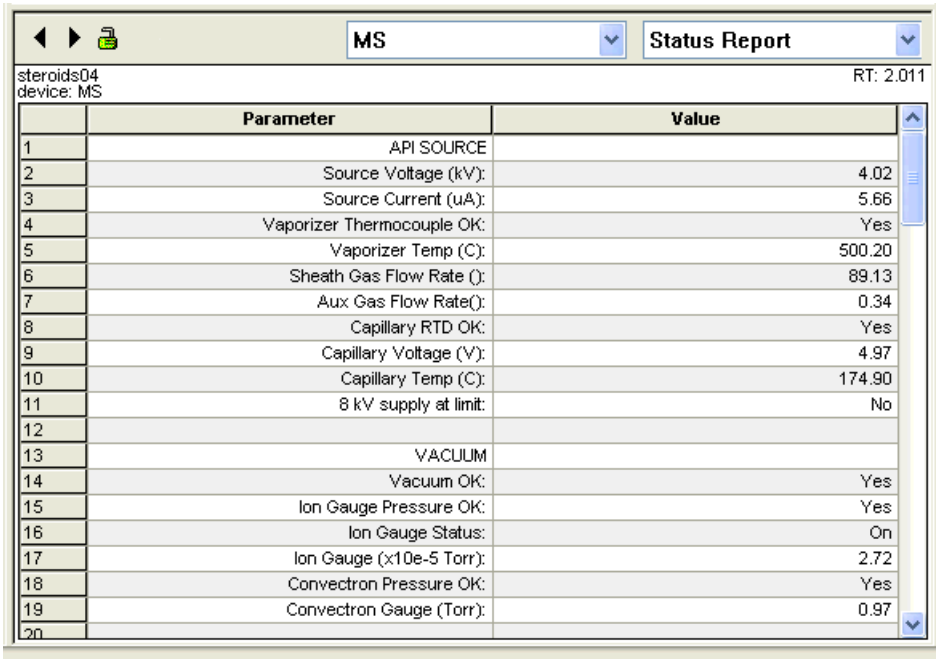
Figure 33. Status plot of the capillary temperature



Status Report

A ***status report*** is a list of status information for all parameters at one retention time. See Figure 34. Use the arrows to move between time points of the chromatogram. Note that status information is acquired less frequently than the chromatogram, so the status information does not always change.

Figure 34. Status report



	Parameter	Value
1	API SOURCE	
2	Source Voltage (kV):	4.02
3	Source Current (uA):	5.66
4	Vaporizer Thermocouple OK:	Yes
5	Vaporizer Temp (C):	500.20
6	Sheath Gas Flow Rate ():	89.13
7	Aux Gas Flow Rate():	0.34
8	Capillary RTD OK:	Yes
9	Capillary Voltage (V):	4.97
10	Capillary Temp (C):	174.90
11	8 kV supply at limit:	No
12		
13	VACUUM	
14	Vacuum OK:	Yes
15	Ion Gauge Pressure OK:	Yes
16	Ion Gauge Status:	On
17	Ion Gauge (x10e-5 Torr):	2.72
18	Convectron Pressure OK:	Yes
19	Convectron Gauge (Torr):	0.97
20		

Review Displays

You can customize the layout of the Review and Explore Results page. The five standard layouts are described in Table 5. Use the Current Review Display list to select a layout.

Table 5. Review displays and their layouts

Display	Layout
Multi Peak View	On the left side of the page are two panes, defaulted to Chromatogram and Spectrum. On the right side of the page is one pane, defaulted to Multi Peak Plot.
Comparison View	Displays three stacked panes, defaulted to Chromatogram, Spectrum, and Peak List.
Peak List View	Displays, side by side, two tall panes, defaulted to Chromatogram and Peak List.
Sample Info View	Displays four panes: one pane in the top row and three small side-by-side panes in the bottom row, defaulted to Chromatogram, Sample Info, Instrument Method List, and Status Plot.
Large Chro View	Displays a large single pane, defaulted to Chromatogram.

Note You can change the contents of the panes, but not their sizes, shapes, and positions.

❖ To change the name of the layout

Select the name in the Review Display list and then type in the new name.

❖ To import a layout

Choose **Files > Layout Configuration > Import Layout Configuration** to display the LCquan Layout Config dialog box. Then, browse to the LCquan config file and click on **Open**. LCquan places the config file in the Imports folder.

❖ To save a layout

Choose **Files > Layout Configuration > Export Layout Configuration** to display the LCquan Config File dialog box. Then, enter a name for the config file and click **OK**. LCquan places the config file in the Exports folder.

Note If you change the properties of a display and save the workbook, the new properties will be active when you reopen the workbook.

Exporting the Active Raw File to Qual Browser

LCquan provides a direct link to Qual Browser from the Review and Explore Results page. Qual Browser provides for more sophisticated qualitative review than what is available in LCquan, which is mainly concerned with quantitative rather than qualitative analysis. With Qual Browser you can perform library searches, formula searches for spectra, background subtraction, and other data manipulation.

❖ To open Qual Browser

Click on the **Qual Browser** button in the viewbar. When you open Qual Browser, LCquan passes the currently selected raw file to it.

Creating a Processing Method

This chapter describes how to create a Processing Method by using the Create Method pages of the Quantitate view. A ***Processing Method*** provides instructions to LCquan on how to perform quantitative analysis on raw data. A Processing Method contains component identification, detection, integration, and calibration information.

Contents

- Opening the Create Method Page and Using the New Method Wizard
- Specifying the Component Identification and Integration Parameters
- Ion Ratio Confirmation of Analytes
- Importing the Components and Levels into the Acquisition Sequence
- Saving the Processing Method
- Importing an Existing Processing Method

Opening the Create Method Page and Using the New Method Wizard

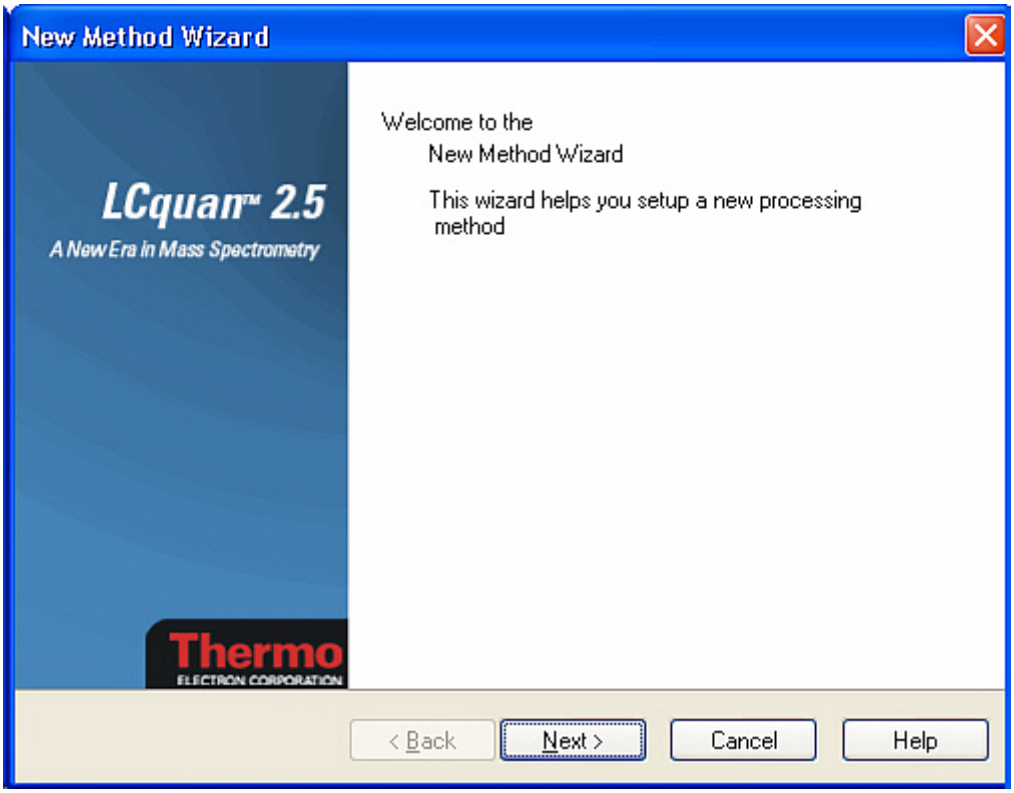
In this example, you need to open the Create Method page of the Quantitate view to construct a Processing Method. When you open this page, the New Method Wizard appears and leads you through the initial steps of creating a method.

❖ To open the Create Method page of the Quantitate view and start the New Method Wizard

1. From any view, click on the **Quantitate** Quantitate button in the LCquan viewbar. The New Method Wizard appears. See Figure 35.
2. Click on **Next** on the Welcome page of the wizard to continue.
3. Select the **Create New Method** option button, the **Initialize with Acquisition Component Names** check box, and click on **Next**.

Note The Initialize With Acquisition Component Names check box is unavailable if you have not yet defined component names.

Figure 35. New Method Wizard – Welcome page



4. Select the **Calibrate Using Internal Standards** option button to specify that you are calibrating by using an internal standard. Then, click on **Next**.
5. Open a raw file from your data set to test the peak detection and integration parameters:

   Note In general, open a raw file that corresponds to a low-concentration calibration standard.

   a. Click on **Browse**.

   b. Browse to find the ***steroids04.raw*** file in the Rawfiles folder in your new workbook. See Figure 36.

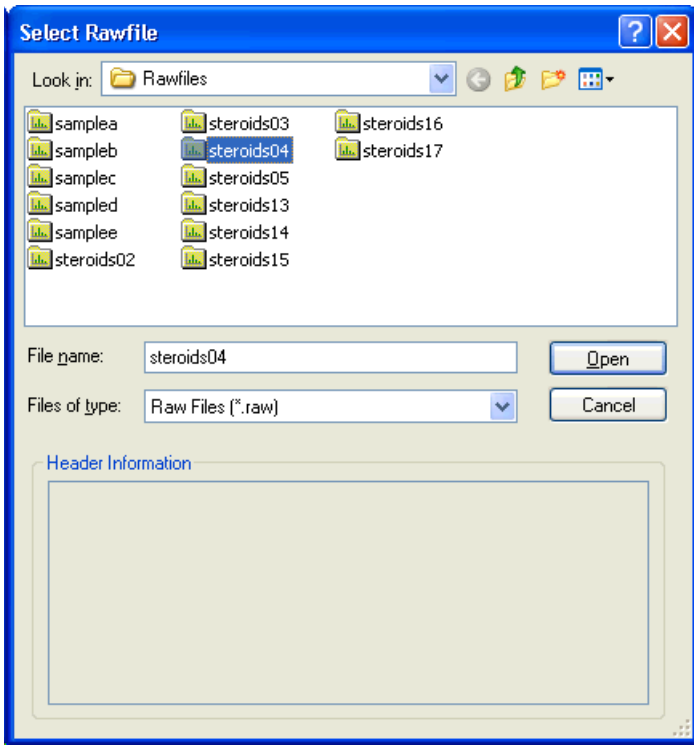
      In this example, the default path is *Xcalibur\QuanRoot\Practice Study\Steroids Workbook\Rawfiles\steroids04.raw.*

   c. Click on **Open**.

   d. Click on **Next**.

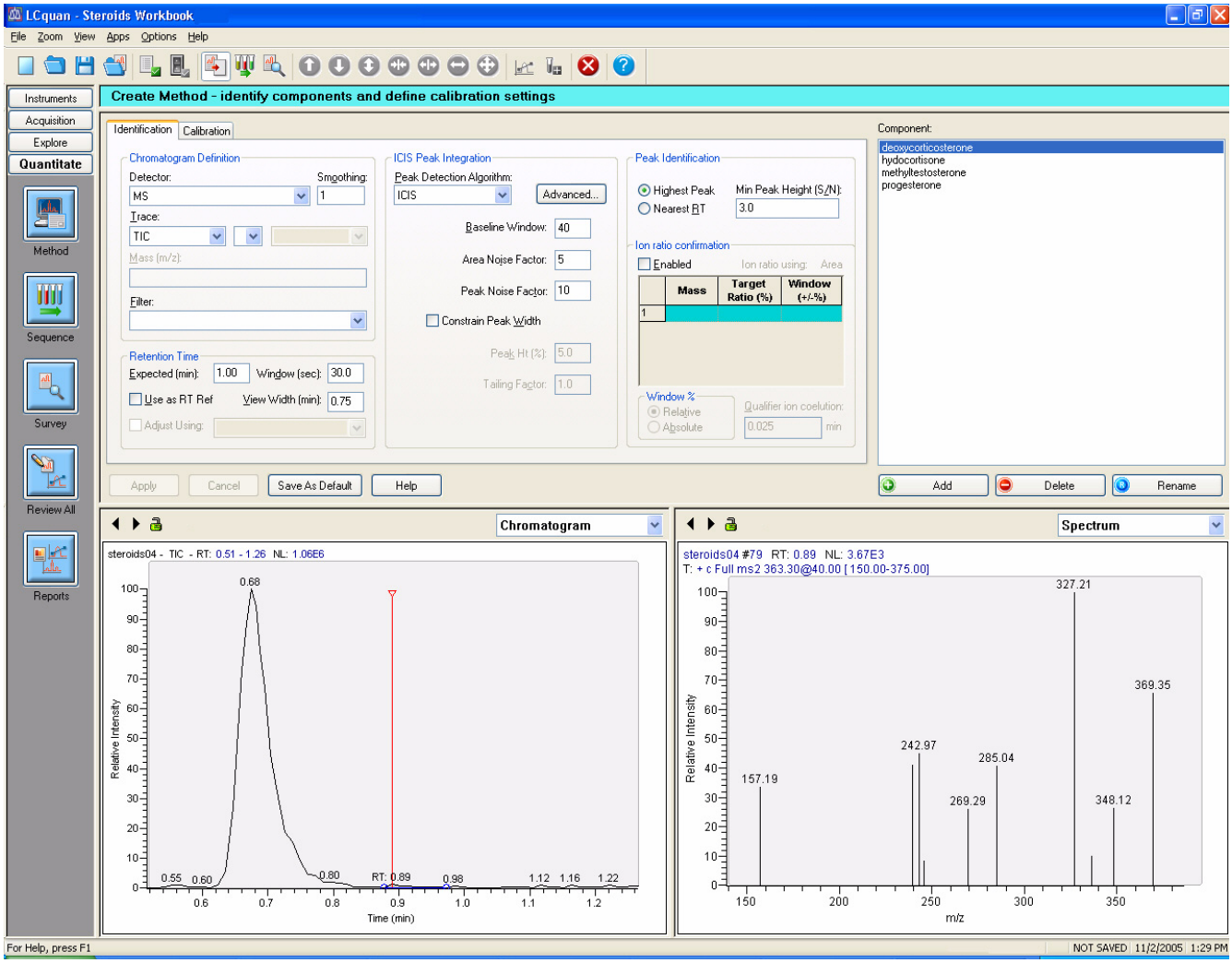
6. Click on **Finish** to exit the New Method Wizard.

Figure 36. Select Rawfile dialog box, showing *steroids04.raw* selected



The Create Method – Identify Components page of the Quantitate view opens. See Figure 37. You are now ready to begin building the Processing Method.

Figure 37. Create Method – Identify Components page of the Quantitate view



Specifying the Component Identification and Integration Parameters

LCquan needs component identification information to associate the target compounds and internal standard with chromatogram peaks. The following topics describe how to specify the identification settings for the target compounds and internal standard:

- Identifying the Internal Standard
- Specifying Calibration Curve Parameters for the Target Compounds
- Entering the Component Identification Parameters and Integrating the Peaks

Identifying the Internal Standard

In the last section, you specified to import the component names and calibration levels from the Acquisition Sequence into the Processing Method. Proceed as follows to identify the internal standard.

❖ **To identify methyltestosterone as the internal standard compound and specify calibration parameters**

1. Select **methyltestosterone** in the Component list.
2. Click on the **Calibration** tab to display the calibration settings.
3. Select Component Type: **ISTD** to specify that methyltestosterone is the internal standard.
4. Enter **0.63** in the ISTD Amount box, and **ng** in the ISTD Units box, to specify that there is 0.63 ng of internal standard. Click on **Apply** to apply the settings. (If necessary, click **OK** to dismiss the warning.)

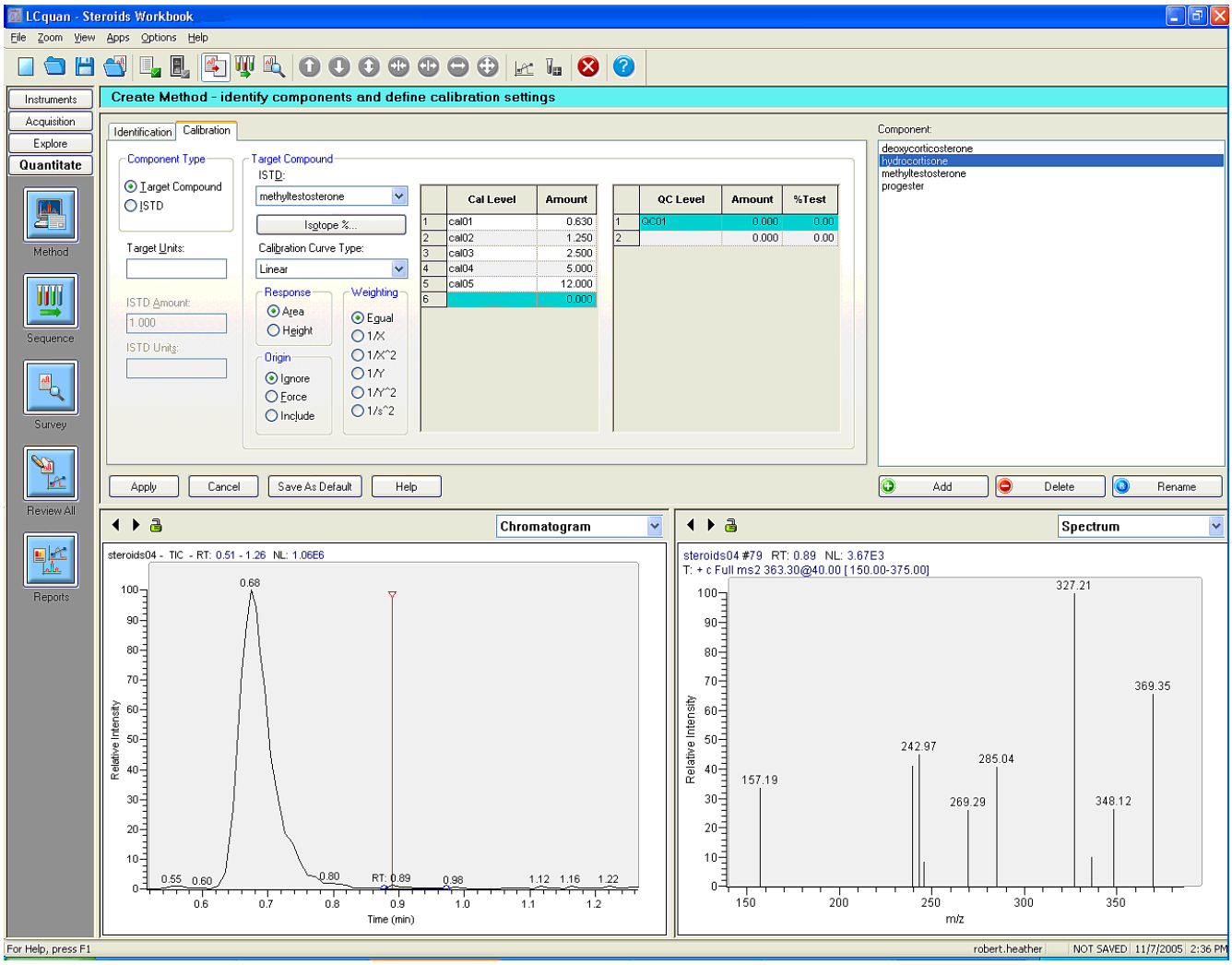
Specifying Calibration Curve Parameters for the Target Compounds

❖ **To specify the calibration curve parameters for the target compounds**

1. Specify the calibration curve parameters for hydrocortisone:
   a. Select **hydrocortisone** in the Component list.
   b. On the Calibration page, select or enter the following:
      - Component Type: **Target Compound**
      - Target Units: **ng**
      - Calibration Curve Type: **Linear**
      - Response: **Area**
      - Origin: **Include**
      - Weighting: **Equal**

c. Click on **Apply** to apply the settings. See Figure 38.

Figure 38. Create Method – Define Calibration Settings page of the Quantitate view, showing the settings for hydrocortisone



2. Specify the calibration curve parameters for deoxycorticosterone:
   a. Select **deoxycorticosterone** in the Component list.
   b. On the Calibration page, select or enter the following:
      - Component Type: **Target Compound**
      - Target Units: **ng**
      - Calibration Curve Type: **Linear**
      - Response: **Area**
      - Origin: **Include**
      - Weighting: **Equal**

c. Click on **Apply** to apply the settings.

3. Specify the calibration curve parameters for progesterone:
   a. Select **progesterone** in the Component list.
   b. On the Calibration page, select or enter the following:
      - Component Type: **Target Compound**
      - Target Units: **ng**
      - Calibration Curve Type: **Linear**
      - Response: **Area**
      - Origin: **Include**
      - Weighting: **Equal**
   c. Click on **Apply** to apply the settings.

Go on to the next topic: "Entering the Component Identification Parameters and Integrating the Peaks".

Entering the Component Identification Parameters and Integrating the Peaks

When LCquan acquires data, it creates unique scan filters according to the type of experiment you specify in the Instrument Method. When you load a raw file, LCquan lists in the Filter combo box the scan filters associated with the raw file. In this example, full scan MS/MS data were acquired on steroids by using alternating product ion scans. To quantitate hydrocortisone, deoxycorticosterone, and progesterone, you need to filter the chromatogram.

You use the Peak Detection Algorithm list box, which is found in the Peak Identification group box, to specify the type of peak detection algorithm (ICIS, Genesis, or Avalon) to be used to analyze the raw data. These algorithms apply smoothing, construct a chromatogram using the scan or mass filters, assign peak numbers, generate a peak list, and determine the peak start and peak end points. All algorithms provide component peak detection and chromatographic peak detection.

❖ To specify the peak detection algorithm, scan filter, and the masses of the significant product ions that you determined in the Explore view for each of the steroids

1. Click on the **Identification** tab to display the Identification page.
2. Enter the settings for methyltestosterone:
   a. Select **methyltestosterone** from the Component list.
   b. Enter or select the following settings:
      - Detector: **MS**
      - Trace: **Mass Range** (specifies a mass range, not TIC, chromatogram)
      - Masses: **267.3, 285.1** (important masses that you determined in the Explore view)
      - Smoothing: **7** (Seven points are to be used in the moving average for smoothing the data.)
      - Peak Detection Algorithm: **Genesis**
      - Filter: **+ c Full ms2 303.30@40 [100.00-310.00]** (scan filter for *m/z* 303 parent mass)
   c. Click on **Apply** to apply the settings.

   LCquan applies the settings to the data in the raw file and automatically displays the resulting filtered chromatogram in the Chromatogram pane.
3. Specify the expected retention time of methyltestosterone:
   a. Inspect the mass chromatogram and note the retention time of methyltestosterone. (Each component peak is labeled with the appropriate retention time.) In this example the methyltestosterone peak is labeled *RT:1.98*.
   b. In the Expected text box, type **1.98** to specify that the retention time of methyltestosterone is 1.98 min.

> **Tip** Two commands in the Chromatogram pane shortcut menu can help you specify the retention time for a component peak: Set Spectrum To Peak Apex and Auto Update Expected RT From Spectrum Marker. (Right-click anywhere in the Chromatogram pane to display the shortcut menu.)
>
> Choose **Auto Update Expected RT From Spectrum Marker** to turn on the automatic updating feature for the expected retention time. When this feature is on, LCquan automatically enters the time indicated by the spectrum marker into the Expected text box in the Retention Time group box. Choosing this command again turns off this feature.
>
> Choose **Set Spectrum To Peak Apex** to automatically place the red spectrum marker at the peak apex and enter the retention time in the Expected box.

c. In the Window text box, type **30.00** to specify that the peak detection time search window is 30.00 s wide (centered about the retention time).

d. Select the **Use As RT Reference** check box to use the retention time of methyltestosterone to adjust for the retention time drift of other components.

e. In the View Width text box, type **0.75** to specify a chromatogram view width of 0.75 min.

f. Click on **Apply** to save the component identification information for methyltestosterone.

4. Integrate the methyltestosterone chromatogram peak:

   a. Click on the chromatogram peak in the Chromatogram pane.

   b. Click on **Apply** to integrate the chromatogram peak.

   LCquan automatically integrates the peak according to the specified parameters. The integrated portion of the peak is shaded gray, and blue identification markers are displayed at the starting and ending points of the peak integration. The blue line that connects the integration markers indicates the baseline. See Figure 39.

   Inspect the integrated peak in the Chromatogram pane and verify that the following are true:

   - The peak has the proper symmetry and the grayed area accurately represents the contribution of the component to the chromatogram.
   - The entire chromatogram peak is displayed in the Chromatogram pane. If necessary, increase the view width by entering a larger value in the View Width text box.
   - The correct scan filter in the Filter text box is matched to the component.

   If the peak has not been identified properly, repeat the procedure outlined in this topic and modify the component identification parameters.

5. Enter the settings for hydrocortisone:

   a. Select **hydrocortisone** from the Component list.

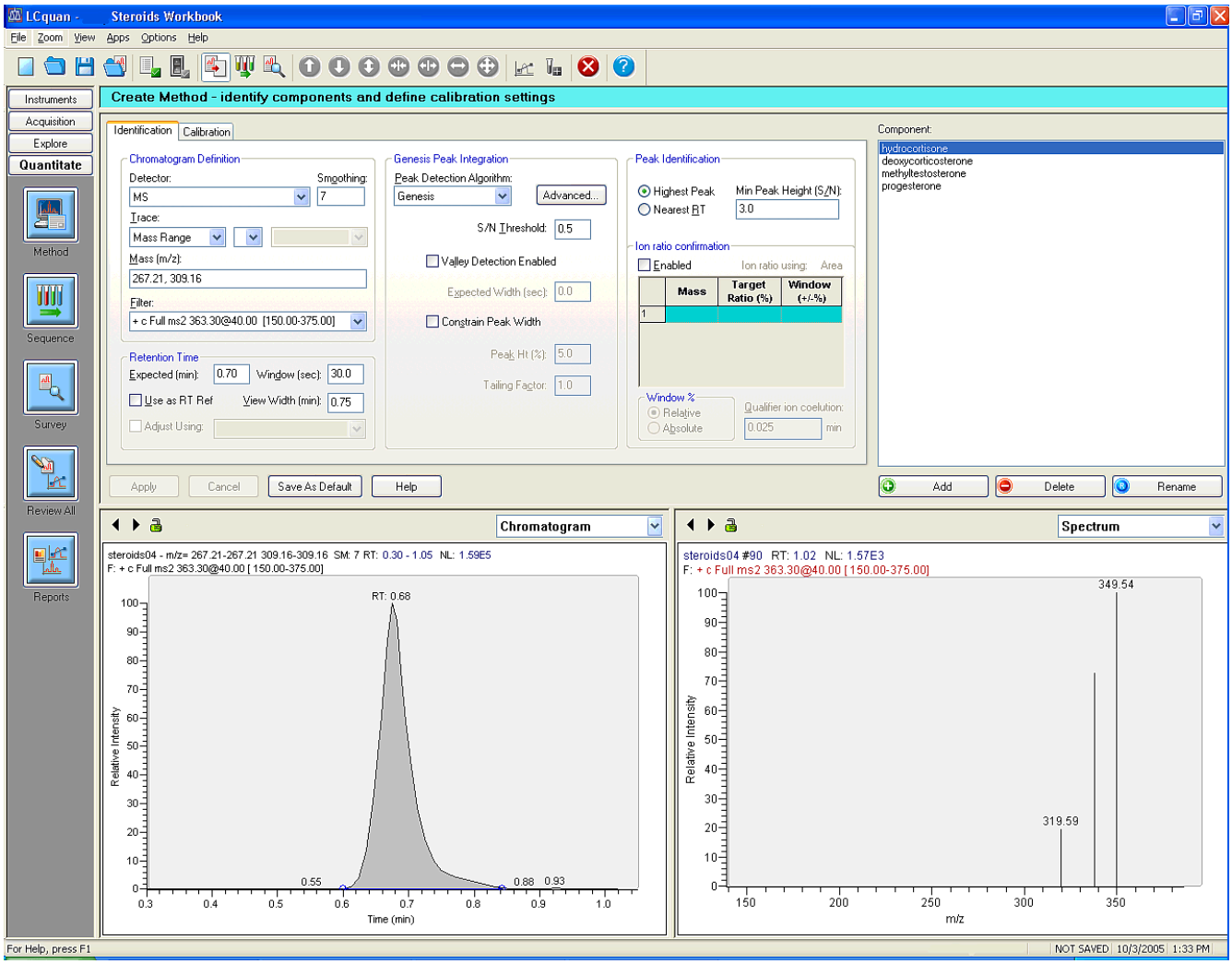
   b. Enter or select the following settings:

      - Detector: **MS**
      - Trace: **Mass Range**
      - Masses: **267.2, 309.2**
      - Smoothing: **7**
      - Peak Detection Algorithm: **Genesis**
      - Filter: **+ c Full ms2 363.30@40 [150.00-375.00]**

   c. Click on **Apply** to apply the settings.

6. Specify the expected retention time of hydrocortisone:
   a. Inspect the mass chromatogram and note the retention time of hydrocortisone. In this example the hydrocortisone peak is labeled *RT:0.67*.
   b. In the Expected text box, type **0.67** to specify that the retention time of hydrocortisone is 0.67 min.
   c. Select the **Adjust Using: methyltestosterone** check box to use the retention time of methyltestosterone to adjust for the retention time drift of hydrocortisone.
7. Integrate the hydrocortisone chromatogram peak:
   a. Click on the chromatogram peak in the Chromatogram pane
   b. Click on **Apply** to integrate the chromatogram peak. See Figure 39.

Figure 39. Create Method – Identify Components page of the Quantitate view, showing the integrated mass chromatogram for hydrocortisone



8. Enter the settings for deoxycorticosterone:
   a. Select **deoxycorticosterone** from the Component list.
   b. Enter or select the following settings:
      - Detector: **MS**
      - Trace: **Mass Range**
      - Masses: **109.0, 253.1, 295.2**
      - Smoothing: **7**
      - Peak Detection Algorithm: **Genesis**
      - Filter: **+ c Full ms2 331.30@40 [100.00-350.00]**
   c. Click on **Apply** to apply the settings.
9. Specify the expected retention time of deoxycorticosterone:
   a. Inspect the mass chromatogram and note the retention time of deoxycorticosterone. In this example the deoxycorticosterone peak is labeled *RT:1.39*.
   b. In the Expected text box, type **1.39** to specify that the retention time of deoxycorticosterone is 1.39 min.
   c. Select the **Adjust Using: methyltestosterone** check box to use the retention time of methyltestosterone to adjust for the retention time drift of deoxycorticosterone.
10. Integrate the deoxycorticosterone chromatogram peak:
    a. Click on the chromatogram peak in the Chromatogram pane
    b. Click on **Apply** to integrate the chromatogram peak.
11. Enter the settings for progesterone:
    a. Select **progesterone** from the Component list.
    b. Enter or select the following settings:
       - Detector: **MS**
       - Trace: **Mass Range**
       - Masses: **255.0**, **279.2, 297.1**
       - Smoothing: **7**
       - Peak Detection Algorithm: **Genesis**
       - Filter: **+ c Full ms2 315.30@40 [100.00-320.00]**
    c. Click on **Apply** to apply the settings.

12. Specify the expected retention time of progesterone:
    a. Inspect the mass chromatogram and note the retention time of progesterone. In this example the progesterone peak is labeled *RT:3.16*.
    b. In the Expected text box, type **3.16** to specify that the retention time of progesterone is 3.16 min.
    c. Select the **Adjust Using: methyltestosterone** check box to use the retention time of methyltestosterone to adjust for the retention time drift of progesterone.
13. Integrate the progesterone chromatogram peak:
    a. Click on the chromatogram peak in the Chromatogram pane.
    b. Click on **Apply** to integrate the chromatogram peak.

> **Note** The ICIS peak detection algorithm has been designed for MS data and has superior peak detection efficiency at low MS signal levels. The Genesis peak detection algorithm has been provided for backwards compatibility with Xcalibur 1.0 studies. The Avalon peak detection algorithm has been designed for UV data. Avalon also supports negative peaks.

You have finished entering the component identification and integration settings for the target compounds and the internal standard. Review the integrated peaks again:

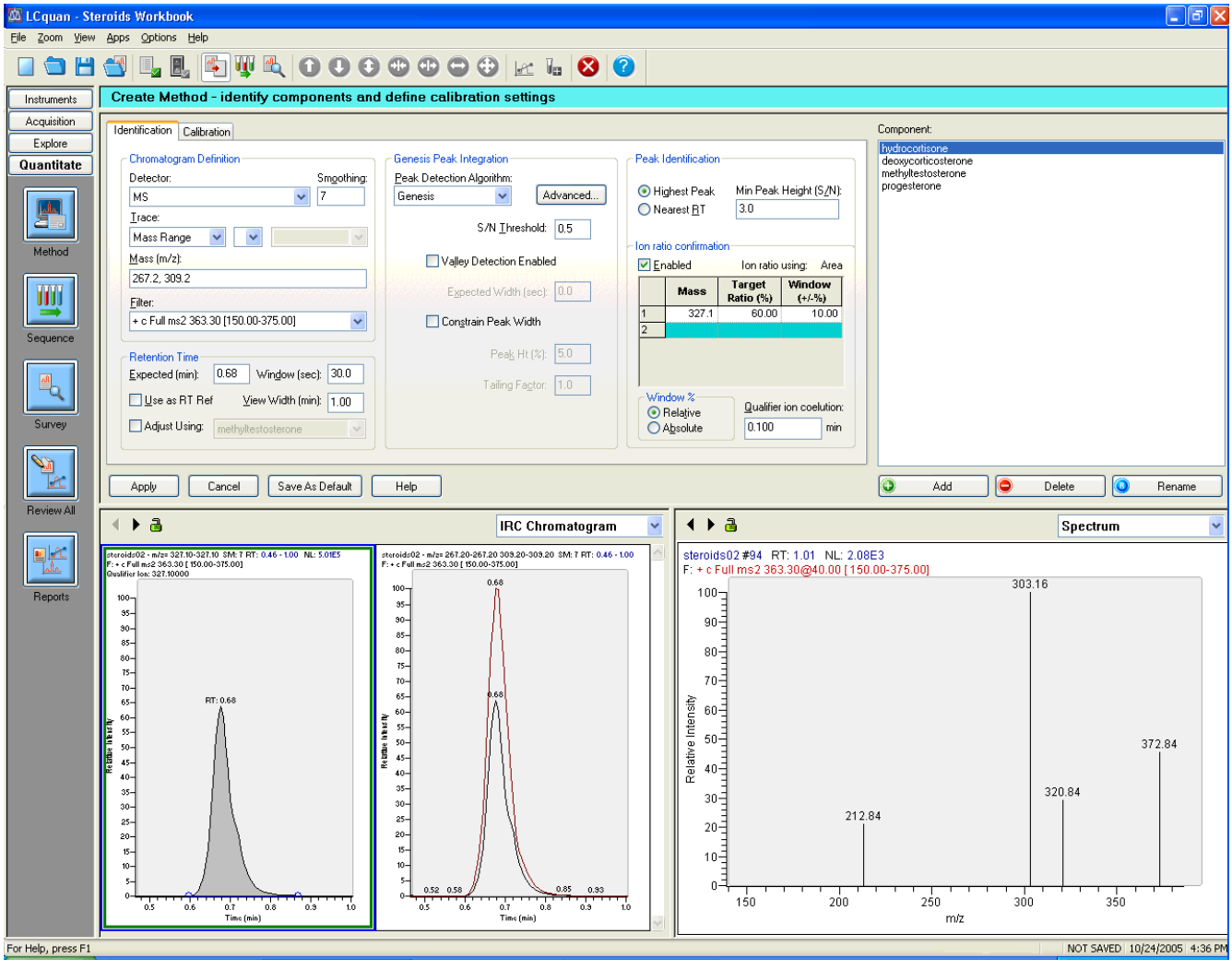
- If the peaks have been integrated correctly, go to the next topic: "Ion Ratio Confirmation of Analytes".
- If there is excessive noise in a peak, unresolved peaks, or peak tailing, return to the topic "Entering the Component Identification Parameters and Integrating the Peaks" on page 77 and change the peak identification and integration parameters.
- If baseline noise is interfering with peak identification or integration, change the settings in the Advanced Parameters dialog box. See the online Help for further information.

Ion Ratio Confirmation of Analytes

Ion ratio confirmation (IRC) allows you to confirm the presence of an analyte by comparing the response of the quantitate ion (or ions) with the responses of one to five qualifier ions. LCquan marks the analyte as Not Found, in the Results List of the Quantitate view, if any of the qualifier ion/quantitate ion response ratios are outside of the upper or lower bounds that you specify, or if the quantitate and qualifier ions do not coelute.

You specify the masses, target area ratios, and area ratio windows of the qualifier ions in the Ion Ratio Confirmation table of the Processing Method. LCquan displays the mass chromatograms of the qualifier and quantitate ions in the IRC Chromatogram pane. In the example shown in Figure 40, LCquan will mark hydrocortisone as Not Found if the area of the *m/z* 327.2 mass chromatogram peak, divided by the area of the *m/z* 309.2+267.2 mass chromatogram peak and multiplied by 100, is less that 50% or greater than 70%. Also, the peak maximum of the qualifier ion chromatogram must be within 0.1 min of the peak maximum of the quantitate ion chromatogram.

Figure 40. Ion ratio conformation settings for hydrocortisone, using *m/z* 327.1 as the qualifier ion



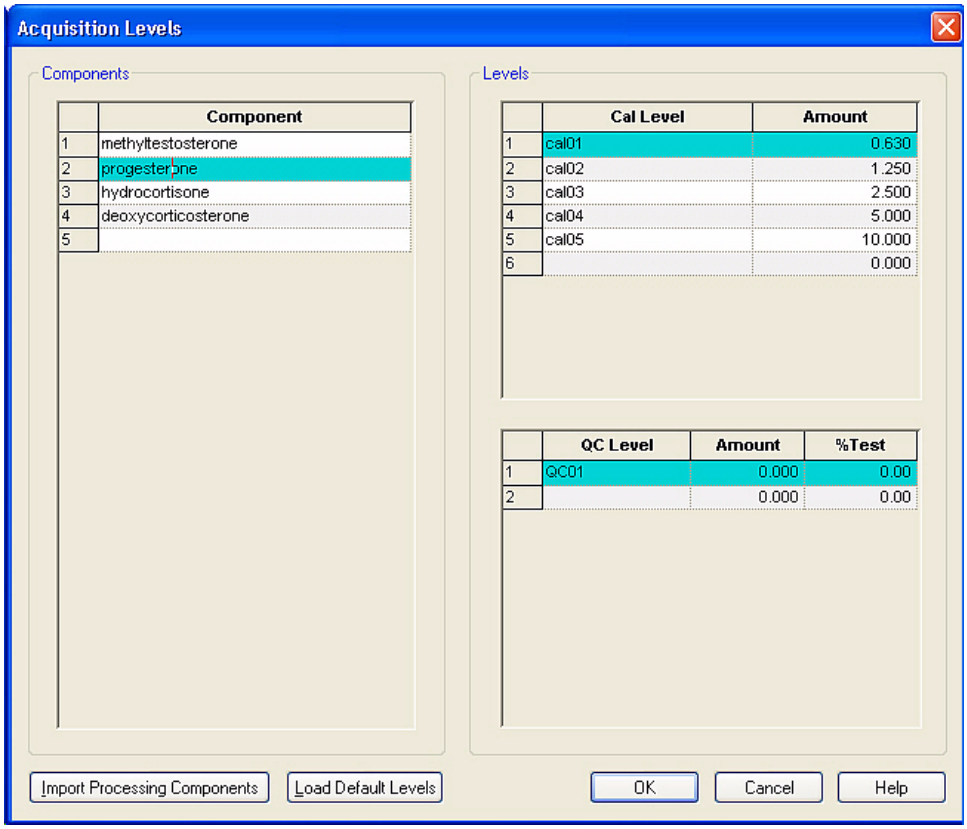
Importing the Components and Levels into the Acquisition Sequence

In the topic "Opening the Create Method Page and Using the New Method Wizard" on page 72, you imported the component names and calibration levels from the Acquisition Sequence into the Processing Method. You can also do the reverse, that is, import the component names and calibration levels from the Processing Method into the Acquisition Sequence. Although it is not necessary to do this for the steroids example, the procedure is described below.

❖ To import the component names and calibration levels into the Acquisition Sequence

1. Click on the **Acquisition** Acquisition button and then the **Setup** button to display the Acquisition Sequence.
2. Right-click on the Sequence grid and select **Standard and QC Levels** from the shortcut menu to display the Acquisition Levels dialog box.
3. Click on **Import Processing Components** Import Processing Components and then on **OK** to import the component names and calibration levels from the Processing Method. See Figure 41.

Figure 41. Acquisition Levels dialog box, showing the steroids components and calibration levels



Saving the Processing Method

When you have finished creating the Processing Method, choose **File > Save** to save the settings in the workbook.

For information on other ways to define a Processing Method in a new workbook, go to the next topic: "Importing an Existing Processing Method". Otherwise, go to Chapter 5, "Creating a Processing Sequence."

Importing an Existing Processing Method

When you are doing routine quantitative analysis experiments, you might save time by importing an existing Processing Method into your current workbook instead of creating a new method. You can then use the method as is or edit it if necessary.

You can import a Processing Method in the following ways:

- By using the New Study Wizard:

  When you create a new workbook, the New Study Wizard gives you the option of importing Instrument Methods, Acquisition Sequences, and processing parameters to initialize the new workbook. You can select to import all of this data from an existing workbook, or you can select to import a Processing Method from an individual legacy file (*.pmd* file) or from an LCquan file (*.lqn* file).

- By using the New Method Wizard in the Quantitate view:

  When you use the New Method Wizard in the Quantitate view, you have the option of importing a previously saved Processing Method. You can import a Processing Method from an individual legacy file (*.pmd* file) or from an LCquan file (*.lqn* file).

- By using the menu command from within the Quantitate view:

  From the Create Method page of the Quantitate view, you can choose **File > Import > Processing Method** to display the Import Quantitation Method dialog box. This dialog box allows you to import a Processing Method from an individual legacy file (*.pmd* file) or from an LCquan file (*.lqn* file).

Creating a Processing Sequence

This chapter describes how to create a Processing Sequence by using the Sequence page of the Quantitate view. A ***Processing Sequence*** provides instructions to LCquan on how to process a batch of raw data files. It consists of a list of sample data files and includes information on sample type and calibration or QC levels.

Contents

- Opening the Create Sequence Page and Using the New Processing Sequence Wizard
- Associating Raw Files with Sample Types
- Reviewing the Processing Sequence
- Further Information: Additional Ways to Define the Processing Sequence
- Further Information: Editing the Processing Sequence

Opening the Create Sequence Page and Using the New Processing Sequence Wizard

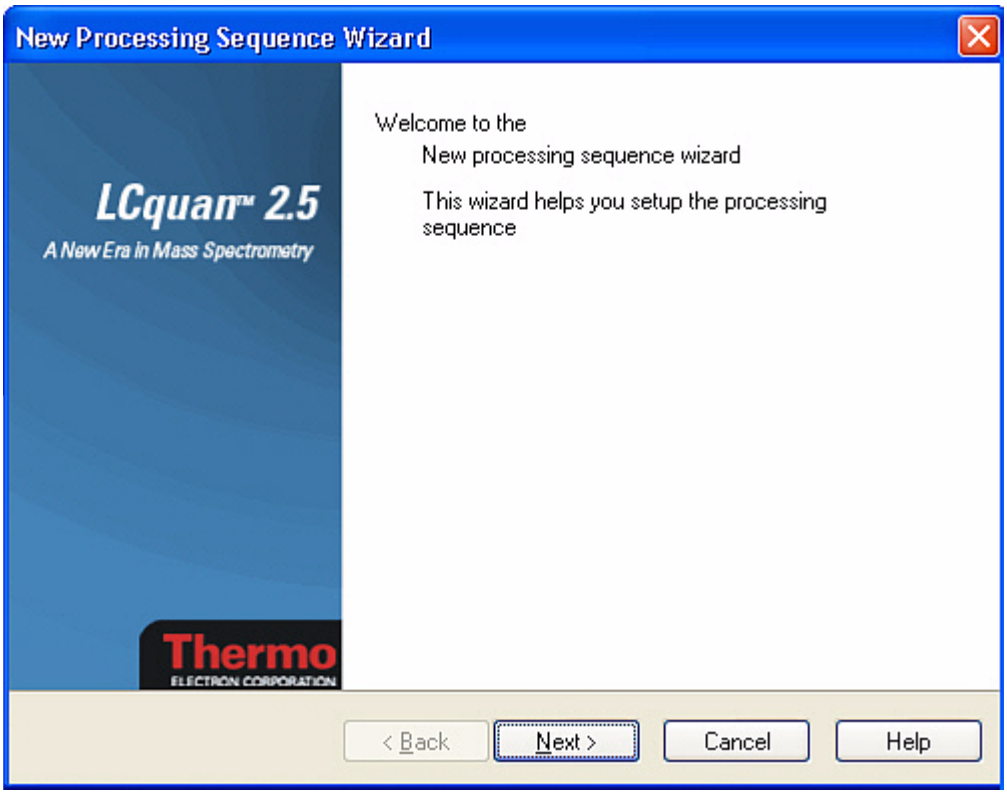
In this example, you use the Create Sequence Page of the Quantitate view to create a new Processing Sequence. When you click on the Sequence icon, LCquan opens the New Processing Method Wizard, unless you have previously created a Processing Sequence, in which case LCquan opens the Create Sequence - Review and Edit page.

❖ To create a new Processing Sequence for the steroids example

1. Click on the **Quantitate** button and then the **Sequence** icon to open the New Processing Sequence Wizard. See Figure 42.
2. Click on **Next** on the Welcome page of the New Processing Sequence Wizard to continue.
3. Select the **Create New Sequence** option button and click on **Next**.
4. Click on **Finish** to exit the New Processing Method Wizard.

The Create Sequence – Select Files page of the Quantitate view opens. You are now ready to begin defining the Processing Sequence.

Figure 42. New Processing Sequence Wizard – Welcome page



Associating Raw Files with Sample Types

When you use LCquan for quantitative analysis, you normally do not need to create a new Processing Sequence in the manner described below. Instead, you copy the Acquisition Sequence from the current workbook to use as the Processing Sequence, or you import an existing sequence from another workbook or legacy file. These techniques allow you to define your Processing Sequence quickly and conveniently. For more on these techniques, refer to "Further Information: Additional Ways to Define the Processing Sequence" on page 95.

The steroids quantitative analysis example outlined in this manual is a special case. You do not need to create an Acquisition Sequence, because the raw data already exists. For practice, you created an Acquisition Sequence in the topic "Creating an Acquisition Sequence for the Steroids Example" on page 30. This Acquisition Sequence associated raw files with calibration levels or unknowns, and specified that there were four components and five calibration levels.

You use the Create Sequence – Select Files page to build a Processing Sequence by associating each raw file in the data set with one of the following sample types: standard, QC, blank, or unknown. You then associate the standards and QCs with the appropriate calibration level or quality control level.

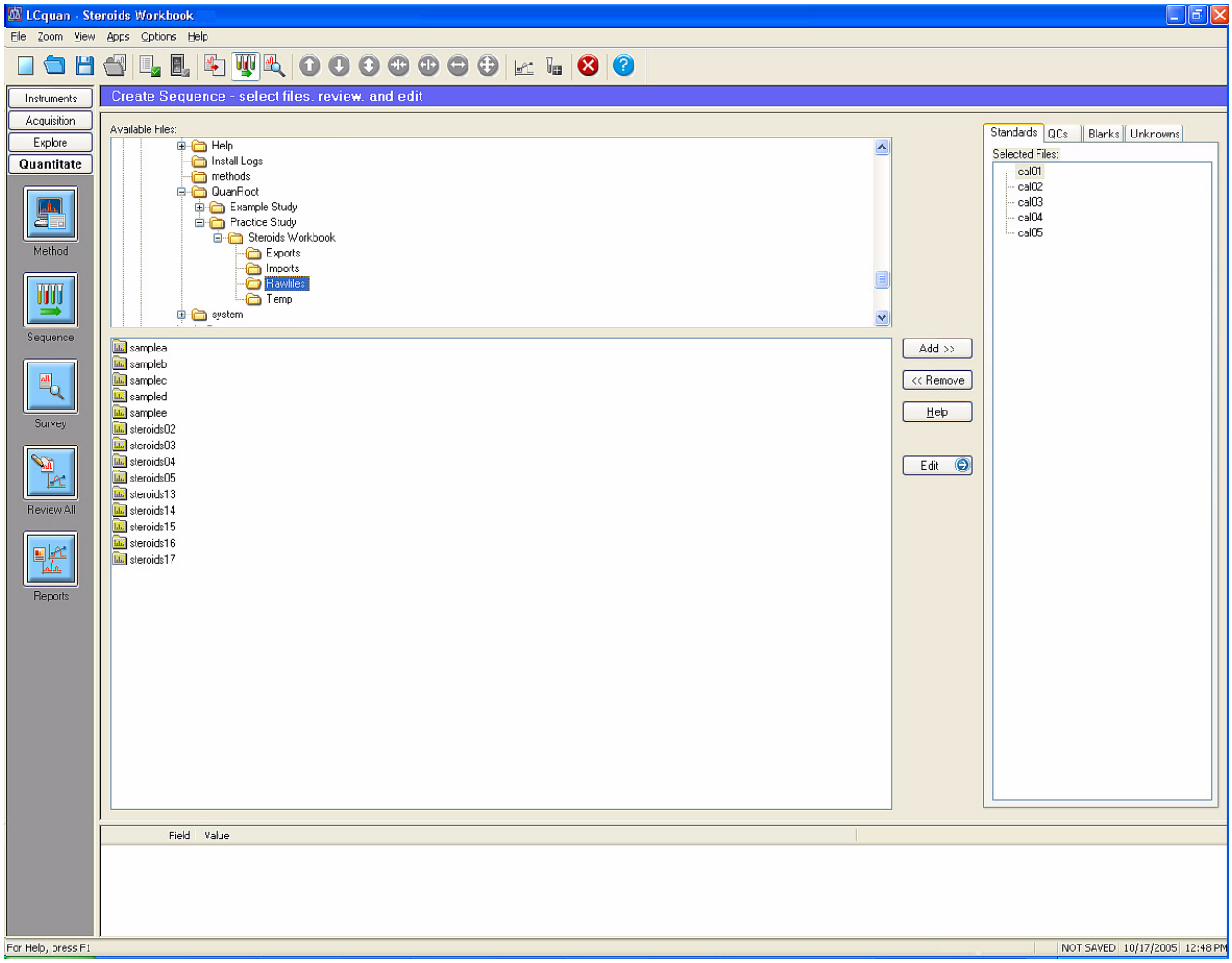
❖ To build a new Processing Sequence for the steroids example

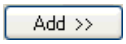
1. Locate the steroids raw data set:
   a. In the directory tree in the Available Files pane, browse to find the *Rawfiles* folder in your current workbook. In this example, the default path is:

      Xcalibur\QuanRoot\Practice Study\Steroids Workbook\Rawfiles.

   b. Click on the *Rawfiles* folder. LCquan displays in the Available Files pane all of the steroids raw files. See Figure 43.

Figure 43. Create Sequence – Select Files page of the Quantitate view, showing the raw data files in the steroids data set



2. Associate the calibration data files with the correct calibration levels:
   a. Ensure that the Standards page of the Selected Files pane is displayed. If the Standards page is not displayed, click on the Standards tab to display it.
   b. In the Standards page, click on **cal01** to select the first calibration level.
   c. In the Data Files pane, click on **steroids05** to select the file.
   d. Click on the **Add** 

button. LCquan adds the selected raw file to the cal01 calibration level in the Selected Files pane.

> **Tip** You can also associate a raw file with a sample type by clicking on the raw file in the Data Files pane and then dragging it to the appropriate level or page in the Selected Files pane.

   e. Associate the raw files and calibration levels as shown in Table 6. The Create Sequence – Select Files page should look like the one shown in Figure 45.

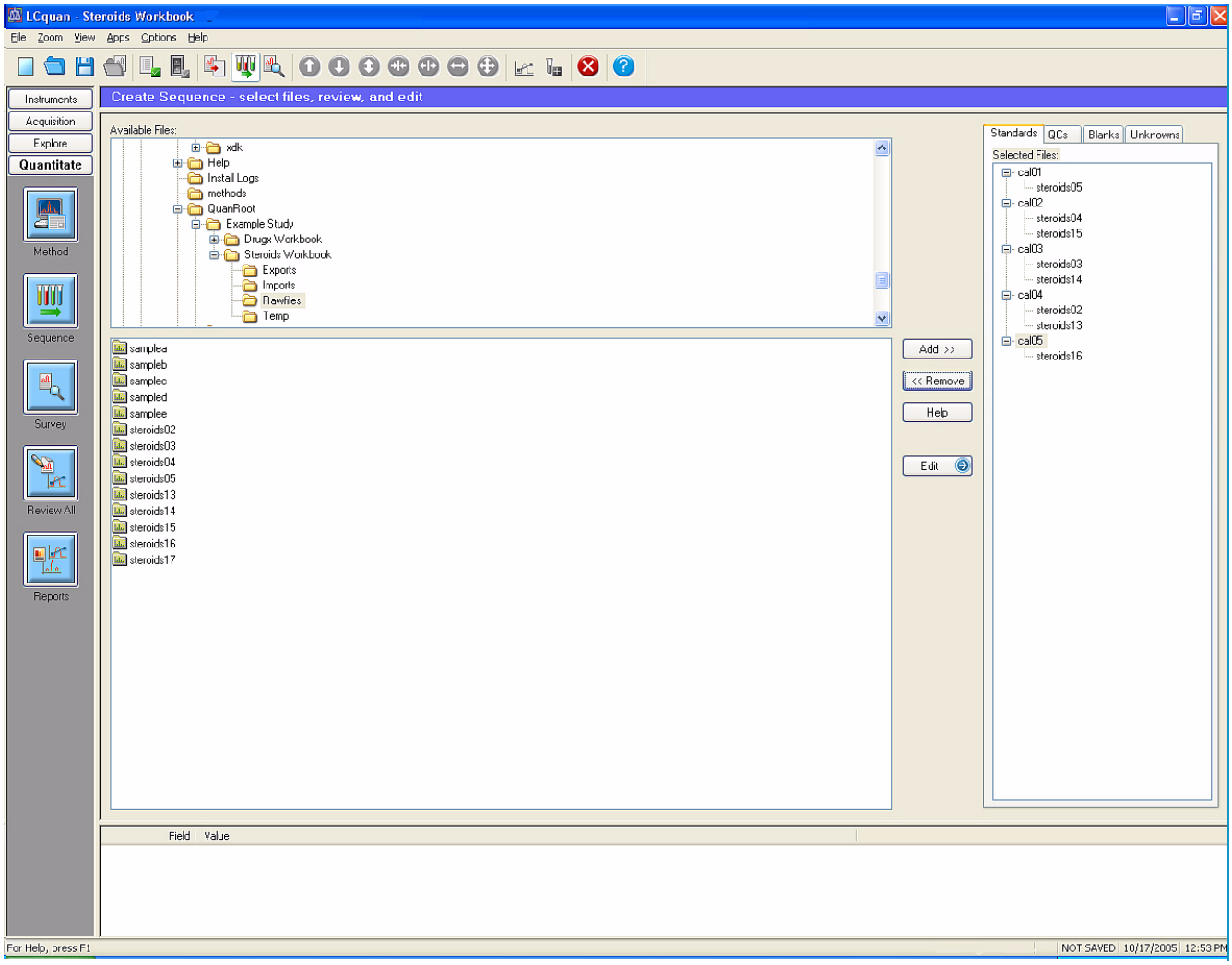
Table 6. Calibration levels and associated raw files

Calibration Level	Raw File
cal01	steroids05
cal02	steroids04 steroids15
cal03	steroids03 steroids14
cal04	steroids02 steroids13
cal05	steroids16

> **Tip** If necessary, you can use the sample type and concentration information in the File Information pane at the bottom of the view to identify the data files.

> **Tip** To display the amount of standard sample associated with a calibration level, right-click on the calibration level in the Selected Files pane and then choose **Show Level Amounts**.

Figure 44. Create Sequence – Select Files page of the Quantitate view, showing the raw files associated with the calibration levels

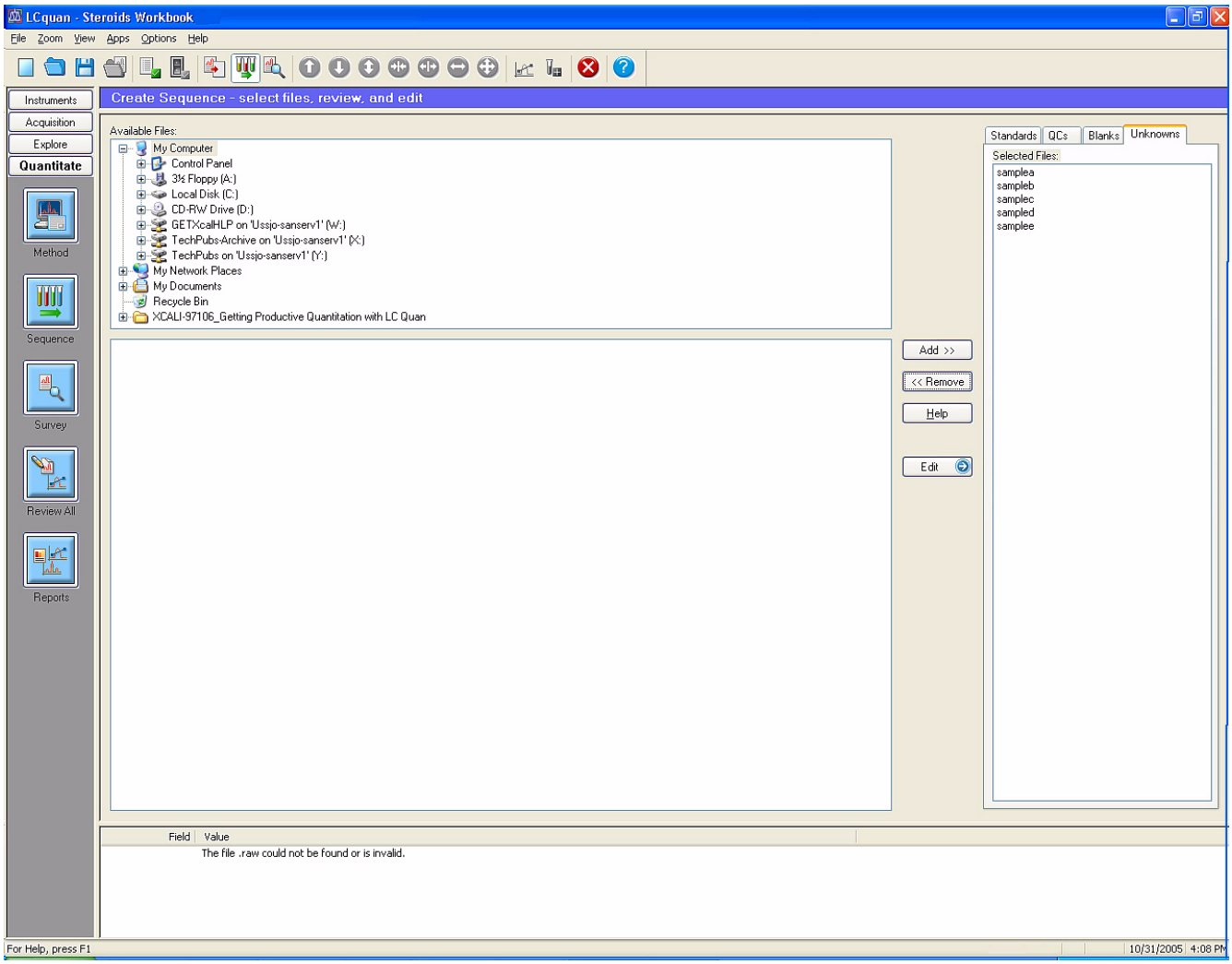


3. Associate *samplea, sampleb, samplec, sampled,* and *samplee* with the unknown sample type:
   a. Click on the **Unknowns** tab in the Selected Files pane.
   b. In the Available Files pane, click on **samplea** to select the file.
   c. Hold down SHIFT and click on **samplee**.
   d. Click on **Add**. LCquan adds the five selected raw files to the Unknowns page of the Selected Files pane. If necessary, delete the blank line above *samplea* by selecting it and then clicking **Remove**.

The Create Sequence – Select Files page should look like the one shown in Figure 45.

Note The steroids data set does not contain any quality control or blank samples. When a data set does contain QCs or blanks, you associate the raw data files to the QC or blank sample type in the same manner as above.

Figure 45. Create Sequence – Select Files page of the Quantitate view, showing the proper association of raw files to the unknown sample type for the steroids example



Reviewing the Processing Sequence

After you associate all of the raw data files with sample types, you need to review the Processing Sequence to ensure that it is correct.

❖ To review the Processing Sequence

1. Click on the **Edit** Edit button to open the Create Sequence – Review and Edit page and to display the new Processing Sequence.
2. Inspect the Processing Sequence and ensure that each raw file in the steroids data set is properly associated with a calibration level or QC level or is defined as an unknown sample. See Figure 46.

Note To correct errors in the Processing Sequence, click on the **Create** button to return to the Create Sequence – Select Files page. Then, use the **Remove** and **Add** buttons to reassociate the raw files. When you are finished, click on the **Edit** button to return to the Create Sequence – Review and Edit page.

Figure 46. Raw files for the steroids example and their calibration levels

	Sample Type	FileName	Level
1	Standard	steroids05	cal01
2	Standard	steroids04	cal02
3	Standard	steroids15	cal02
4	Standard	steroids03	cal03
5	Standard	steroids14	cal03
6	Standard	steroids02	cal04
7	Standard	steroids13	cal04
8	Standard	steroids16	cal05
9	Unknown	samplea	NA
10	Unknown	sampleb	NA
11	Unknown	samplec	NA
12	Unknown	sampled	NA
13	Unknown	samplee	NA

When you are satisfied that the Processing Sequence for the steroids example is correct, do one of the following:

- If you want more information on Processing Sequences before continuing, go to the next sections: "Further Information: Additional Ways to Define the Processing Sequence" on page 95 and "Further Information: Editing the Processing Sequence" on page 97.
- If you want to process the steroids raw data files, go to Chapter 6, "Processing the Raw Files and Reviewing the Analytical Results."

Further Information: Additional Ways to Define the Processing Sequence

Usually, instead of creating a new Processing Sequence, it is more convenient to use an existing Processing or Acquisition Sequence as your Processing Sequence. You can do this in either of two ways as follows:

- Importing an Existing Sequence
- Copying the Acquisition Sequence

Importing an Existing Sequence

You can import an existing sequence into your current workbook in the following ways:

- By using the New Study Wizard:

  When you create a new workbook, the New Study Wizard gives you the option of importing Instrument Methods, Acquisition Sequences, and processing parameters to initialize the new workbook. You can select to import all of this data from an existing workbook, or you can select to import an Acquisition Sequence from an individual legacy file (*.sld, .ipp,* or *.csv* file) or from an LCquan file (*.lqn* file).

  If you import an Acquisition Sequence from an individual legacy file into a workbook that does not contain a Processing Sequence, LCquan uses the same sequence for the Acquisition Sequence and the Processing Sequence in the current workbook.

- By using the New Processing Sequence Wizard in the Quantitate view:

  When you use the New Processing Sequence Wizard, you have the option to import a previously saved sequence. You can import a sequence from an individual legacy file (*.sld, .ipp,* or *.csv* file) or from an LCquan file (*.lqn* file).

- By using menu commands from within the Quantitate view:

  From the Create Sequence pages of the Quantitate view, you can choose **File > Import > Processing Sequence > From File** to display the Import Sequence File dialog box. This dialog box allows you to import a sequence from an individual legacy file (*.sld, .ipp,* or *.csv* file) or from an LCquan file (*.lqn* file).

  You can also right-click anywhere within the Create Sequence – Review and Edit page and choose **Import Processing Sequence > From File** from the shortcut menu to display the Import Sequence File dialog box.

Note The level names in the Processing Sequence must match those defined in the Processing Method:

- If the calibration or quality control level names in the sequence are different from those in the Processing Method, LCquan displays the Standard Level Names Association dialog box or the QC Level Names Association dialog box. You can use these dialog boxes to associate the level names in the sequence with the level names in the Processing Method.
- If the imported sequence contains calibration or QC levels but the Processing Method does not, LCquan displays a warning box to inform you. It then displays the Sample Type Assignment dialog box. You can select to change the sample type of these samples to *unknown* in the Processing Sequence or to discard the samples that have these levels in the Processing Sequence.

Copying the Acquisition Sequence

If an Acquisition Sequence already exists in the current workbook, you can use it to define the Processing Sequence in the following ways:

- By using menu commands from within the Quantitate view:

  From the Create Sequence pages of the Quantitate view, you can choose **File > Import > Processing Sequence > Copy From Acquisition Sequence** to copy the Acquisition Sequence from the current workbook into the Processing Sequence.

  You can also right-click anywhere within the Create Sequence – Review and Edit page and choose **Import Processing Sequence > Copy From Acquisition Sequence** from the shortcut menu to copy the Acquisition Sequence.

- By using the Acquisition Sequence History in the Create Sequence page:

  If you have already acquired data in the workbook, the Acquisition Sequence (or Sequences) that was used is listed in the Acquisition Sequence History list. You can copy an Acquisition Sequence by selecting a sequence from the list and then dragging and dropping it into the Processing Sequence. You can hold down the CTRL key to select multiple sequences.

If needed, you can edit an imported or copied sequence. Refer to the next topic: "Further Information: Editing the Processing Sequence".

Further Information: Editing the Processing Sequence

You can use the Create Sequence – Review and Edit page to alter the Processing Sequence by removing or adding raw sample files, changing sample types, and modifying the arrangement of the sequence as follows:

- Removing raw files from the Processing Sequence:

  To select the row in the sequence that you want to delete, click the row number. Right-click the sequence grid, and choose **Delete Selected Samples** from the shortcut menu.

- Adding raw files to the Processing Sequence:

  Select the row in the sequence above which you want the new row (sample) to be located. Right-click the sequence, and then choose **Add Sample** from the shortcut menu. In the Select Rawfile dialog box, select the files you want to add, and then click **Open**. LCquan obtains the sample information from the selected files and adds the samples to the sequence.

- Changing the sample type:

  Click the Sample Type box to display the arrow, and then select the new sample type from the list.

- Changing a range of cells:

  You can fill selected columns of selected rows with duplicate text entries or appropriately sequenced number entries. Select the row that you want to copy, right-click the sequence grid, and then choose **Fill Down** from the shortcut menu. In the Sequence Fill Down dialog box, specify the columns to be included and the range of rows.

- Modifying the arrangement of the Processing Sequence:

  You can modify the arrangement of the sequence by using shortcut menu commands. For example, you can change the sorting order of the sequence. Right-click in the sequence to display the shortcut menu.

Refer to the online Help for additional information about editing the Processing Sequence.

Processing the Raw Files and Reviewing the Analytical Results

This chapter describes how to process the raw steroids data by using the Processing Method and the Processing Sequence that you created in the previous chapters. It also explains how to review the results for each component in each raw file and how to produce reports.

Contents

- Processing the Raw Files
- Reviewing the Calibration Curve and QCs
- Reviewing All Results
- Creating and Reviewing Reports
- Locking the Workbook
- Saving the Workbook and Exiting LCquan

Processing the Raw Files

LCquan processes the raw files in the Processing Sequence when you open the Survey page, the Review All Results page, or the Review Reports page of the Quantitate view. LCquan uses the peak detection and integration parameters in the Processing Method to detect and integrate peaks for all components. It then uses the calibration parameters in the Processing Method to build a calibration curve with which it quantitates the QCs and unknowns.

Note You need to define a Processing Method and a Processing Sequence before you can open the Survey page, the Review All Results page, or the Review Reports page.

Although you can review the results in any order, in this example you first review the calibration standards.

Reviewing the Calibration Curve and QCs

You use the Survey page of the Quantitate view to review and rework the calibration standards. The process of reviewing and reworking the calibration standards involves the following steps:

1. Opening the Survey Page
2. Reviewing the calibration standards
3. Excluding a Calibration Standard from the Calibration Curve (optional)
4. Modifying the Peak Detection and Integration Settings (optional)
5. Integrating Peaks Manually (optional)

Opening the Survey Page

After you create the Processing Sequence, you are ready to review the calibration curves.

The Create Sequence – Review and Edit page should be displayed. (See "Reviewing the Processing Sequence" on page 94.) To process the raw files and open the Survey page, click on the **Survey** button in the viewbar. See Figure 47.

The Survey page includes (clockwise from the top) the Result List, the Component List, the Calibration Curve pane, the ISTD Chromatogram pane, and the Chromatogram pane.

The ***Result List*** contains the peak integration results. It has three display tabs: All, Standards, and QCs. Clicking on the All tab displays the results for all of the standards and QCs. The Result List also uses a system of color shading to indicate the status of individual samples. Refer to Table 7.

Figure 47. Survey page of the Quantitate view, showing the results for hydrocortisone

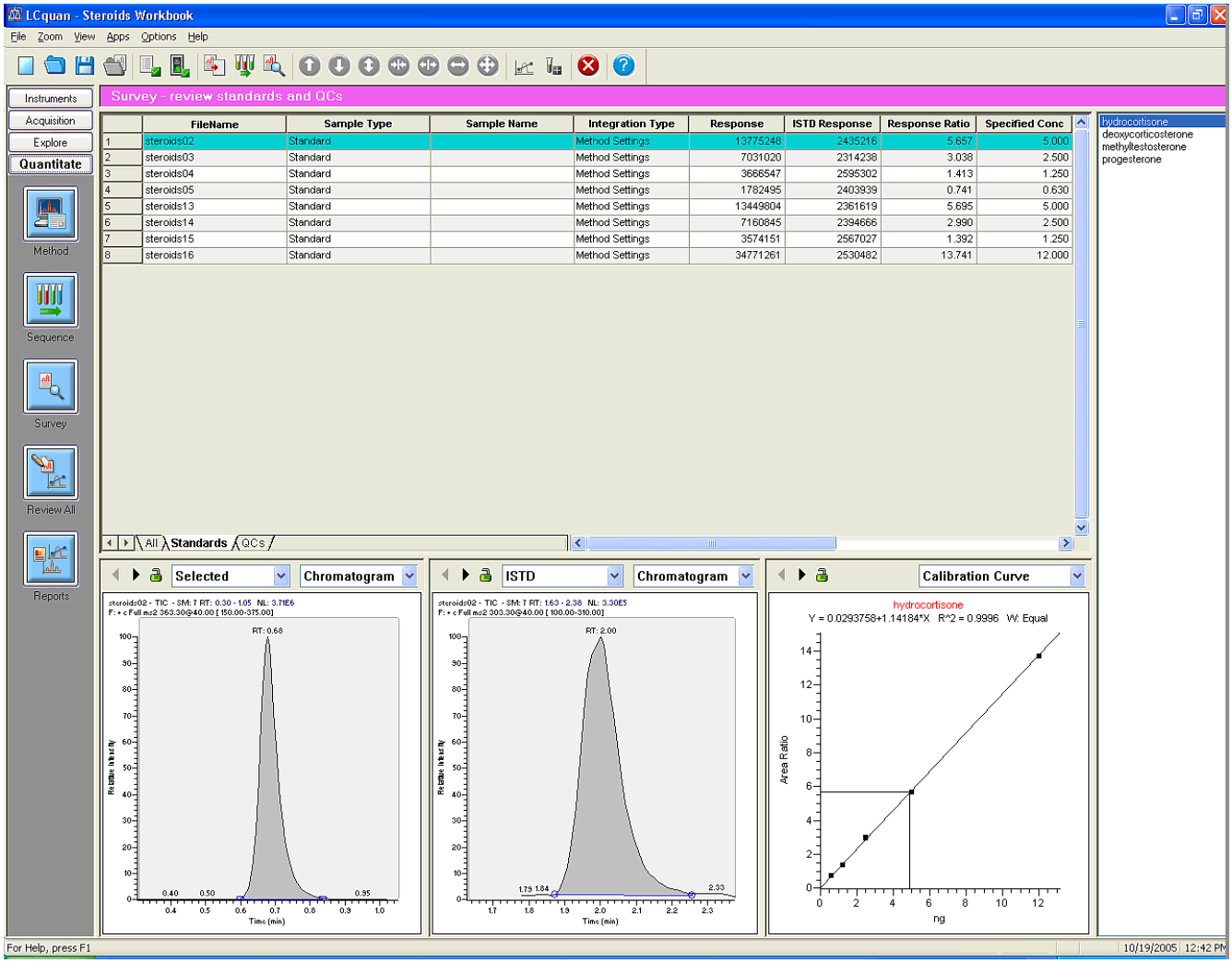

LCquan - Steroids Workbook
File Zoom View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Method
Sequence
Survey
Review All
Reports
Survey - review standards and QCs
FileName Sample Type Sample Name Integration Type Response ISTD Response Response Ratio Specified Conc
1 steroids02 Standard Method Settings 13775248 2435216 5.657 5.000
2 steroids03 Standard Method Settings 7031020 2314238 3.038 2.500
3 steroids04 Standard Method Settings 3666547 2595302 1.413 1.250
4 steroids05 Standard Method Settings 1782495 2403939 0.741 0.630
5 steroids13 Standard Method Settings 13449804 2361619 5.695 5.000
6 steroids14 Standard Method Settings 7160845 2394666 2.990 2.500
7 steroids15 Standard Method Settings 3574151 2567027 1.392 1.250
8 steroids16 Standard Method Settings 34771261 2530482 13.741 12.000
hydrocortisone
deoxycorticosterone
methyltestosterone
progesterone
All Standards QCs
Selected Chromatogram
ISTD Chromatogram
Calibration Curve
RT: 0.68
RT: 2.00
hydrocortisone
Y = 0.0293758+1.14184*X R^2 = 0.9996 W: Equal
Area Ratio
ng
For Help, press F1
10/19/2005 12:42 PM

Table 7. Sample status and corresponding row color in Result List

Sample Status	Row Color
Peak Not Found	Dark Pink
Sample Excluded	Dark Pink
QC Failed	Orange
Response High	Pale Orange
Response Low	Pale Orange
Calculated Amount Is Negative	Yellow
Calculated Amount is Undefined	Orange

The ***Component List*** contains the components that are defined in the current Processing Method. When you select a component in the Component List, all of the other page elements are updated to display the results for that component. The ***Calibration Curve pane*** displays a plot of area ratio (or area) versus concentration (or amount) for the selected component. The ***ISTD Chromatogram pane*** displays a plot of relative intensity versus retention time of the internal standard in the current sample (if available). Finally, the ***Chromatogram pane*** displays a plot of relative intensity versus retention time of the selected component in the current sample.

❖ To review the calibration standards

Note The steroids data set does not contain QC samples.

1. In the Component List on the right side of the page, select the ***hydrocortisone*** target compound. LCquan automatically updates the Result List, the Chromatogram and ISTD Chromatogram panes, and the Calibration Curve pane.
2. Ensure that the calibration standards are displayed in the Result List. If necessary, click on the Standards tab at the bottom of the Result List to display the calibration standards.
3. In the Calibration Curve pane, inspect the calibration curve at all concentrations. When you process your own data, evaluate the calibration curve according to the criteria used in your laboratory.
4. Inspect the calibration curve at low concentrations:
   a. Click and drag the mouse diagonally across the low concentration data points in the calibration curve. See Figure 48.
   b. Release the mouse button. LCquan displays only the selected data points. See Figure 49.

Figure 48. Calibration curve, showing the selection of the low concentration data points

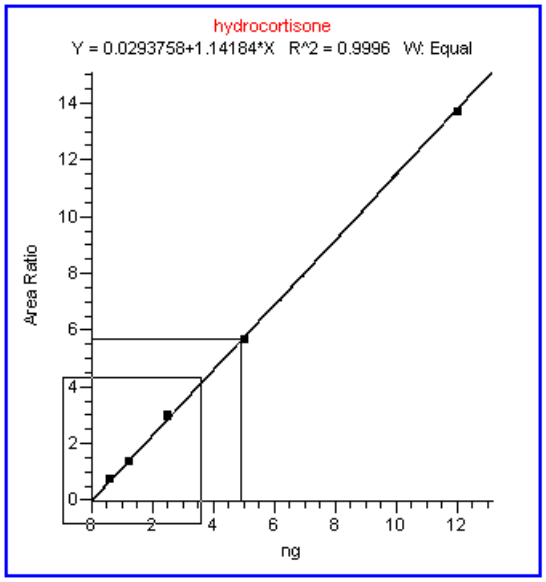
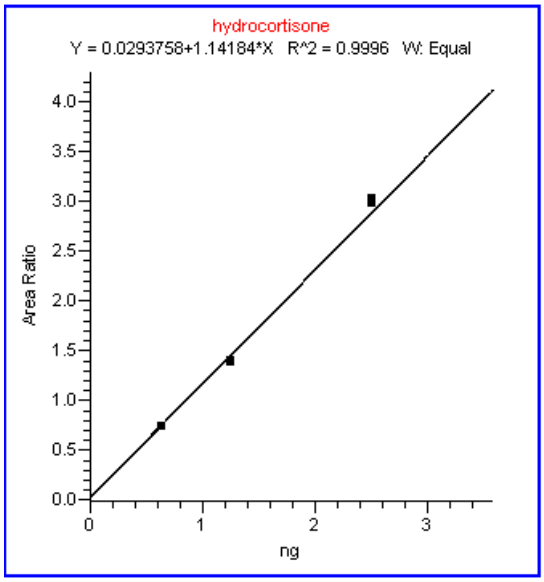


Figure 49. Calibration curve, showing only the low concentration data points



5. Reset the scaling of the X- and Y-axes of the calibration curve to full scale by clicking on the Reset Scaling button.

Tip You can change the calibration curve parameters, including curve type and level information, by using the Calibration Settings dialog box. To open the Calibration Settings dialog box, right-click in the Calibration Curve pane to display a shortcut menu. Then, choose **Calibration Settings** from the shortcut menu.

6. Inspect the Result List:
   a. Check the entries in the Result List for peak detection and integration problems. (Use the scroll bar at the bottom of the list to see all of the entries.)
   b. Ensure that the data files correspond to the correct levels and sample types.
7. Select the first data file by clicking on the first row of the Result List.
8. Inspect the target compounds and internal standard peaks:
   a. Inspect the component peak in the Chromatogram pane:
      i. Ensure that LCquan found the peak. LCquan shades the peak it finds gray and marks the starting and ending points of the peak with integration markers.
      ii. Ensure that the shaded area accurately represents the contribution of the component to the chromatogram.
   b. Inspect the internal standard peak in the ISTD Chromatogram pane:
      i. Ensure that LCquan found the peak. LCquan shades the peak it finds gray and marks the starting and ending points of the peak with integration markers.
      ii. Ensure that the shaded area accurately represents the contribution of the internal standard to the chromatogram.

Tip In the Chromatogram pane and in the ISTD Chromatogram pane, you can use the toolbar buttons to zoom in and out along the X- and Y-axes to show more detail. See the online Help for a description of each toolbar button.

Alternatively, you can use the arrow keys on the keyboard to zoom. The left arrow key zooms in along the X-axis by a factor of two (2). The right arrow key zooms out along the X-axis by a factor of two (after you have zoomed in). Similarly, the up arrow zooms in along the Y-axis by a factor of two and the down arrow zooms out.

- If you want to exclude a calibration standard from the calibration curve, go to the next topic: "Excluding a Calibration Standard from the Calibration Curve".
- If you want to modify the peak detection and integration settings that you defined in the Create Method – Identify Components page, go to the topic: "Modifying the Peak Detection and Integration Settings" on page 109.
- If you want to integrate a peak manually, go to the topic: "Integrating Peaks Manually" on page 110. You can manually change the integration starting and ending points and the baseline of the peak.

9. Inspect the remaining files in the Result List:
   a. Select the next data file by clicking on the next row in the Result List.
   b. Repeat step 8 for each file.

10. Repeat steps 8 and 9 for the remaining target compounds, ***deoxycorticosterone*** and ***progesterone***.

    The calibration curves for hydrocortisone, deoxycorticosterone, and progesterone are shown in Figure 50.

11. Review and rework the internal standard results as you did for the target compound:

    a. In the Component List, select the internal standard, ***methyltestosterone***. LCquan automatically updates the Result List and the Chromatogram pane. LCquan does not display a chromatogram in the ISTD Chromatogram pane for this component but displays the response for each internal standard sample in the Calibration Curve pane.

    b. Check the entries in the Result List for peak detection and integration problems.

    c. Inspect the chromatogram in the Chromatogram pane for each file:

        i. Ensure that LCquan found the peak.

        ii. Ensure that the shaded area accurately represents the contribution of the component to the chromatogram.

        - If you want to modify the peak detection and integration settings that you defined in the Create Method - Identify Components page, go to the topic: "Modifying the Peak Detection and Integration Settings" on page 109.

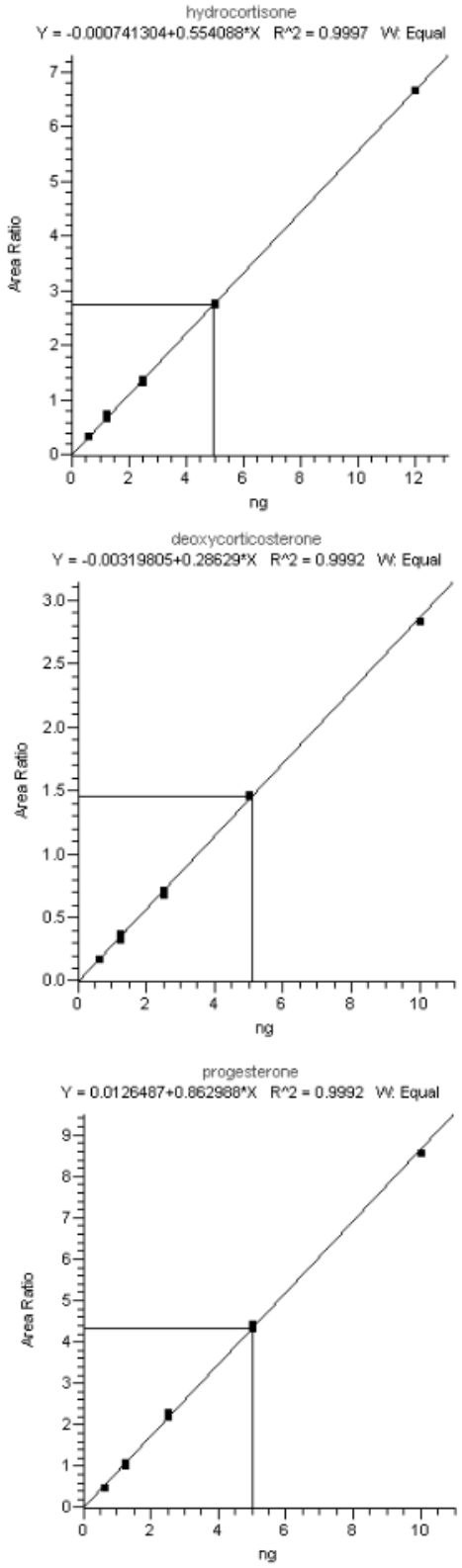
        - If you want to integrate a peak manually, go to the topic: "Integrating Peaks Manually" on page 110. You can manually change the integration starting and ending points and the baseline of the peak.

When you are satisfied that LCquan detected and integrated the peaks properly, you are ready to review all of the results:

- If you want further information about reworking the data, refer to the following topics: "Excluding a Calibration Standard from the Calibration Curve" on page 107, "Modifying the Peak Detection and Integration Settings" on page 109, and "Integrating Peaks Manually" on page 110.

- If you want to review all of the results, go to "Reviewing All Results" on page 112.

Figure 50. Calibration curves for the steroids example



Excluding a Calibration Standard from the Calibration Curve

LCquan allows you to exclude specific calibration standards from the calibration curve. Of course, you should follow the procedures of your laboratory for proper treatment of calibration data.

You can exclude calibration data in the following ways:

- By using the Exclude column of the Result List:

  Ensure that the **Standards** tab or the **All** tab is selected at the bottom of the Result List. For each standard that you want to exclude from the calibration curve, click in the check box in the Exclude column of its row. (To include the standard again, clear the check box.)

- By using the Exclusion List:

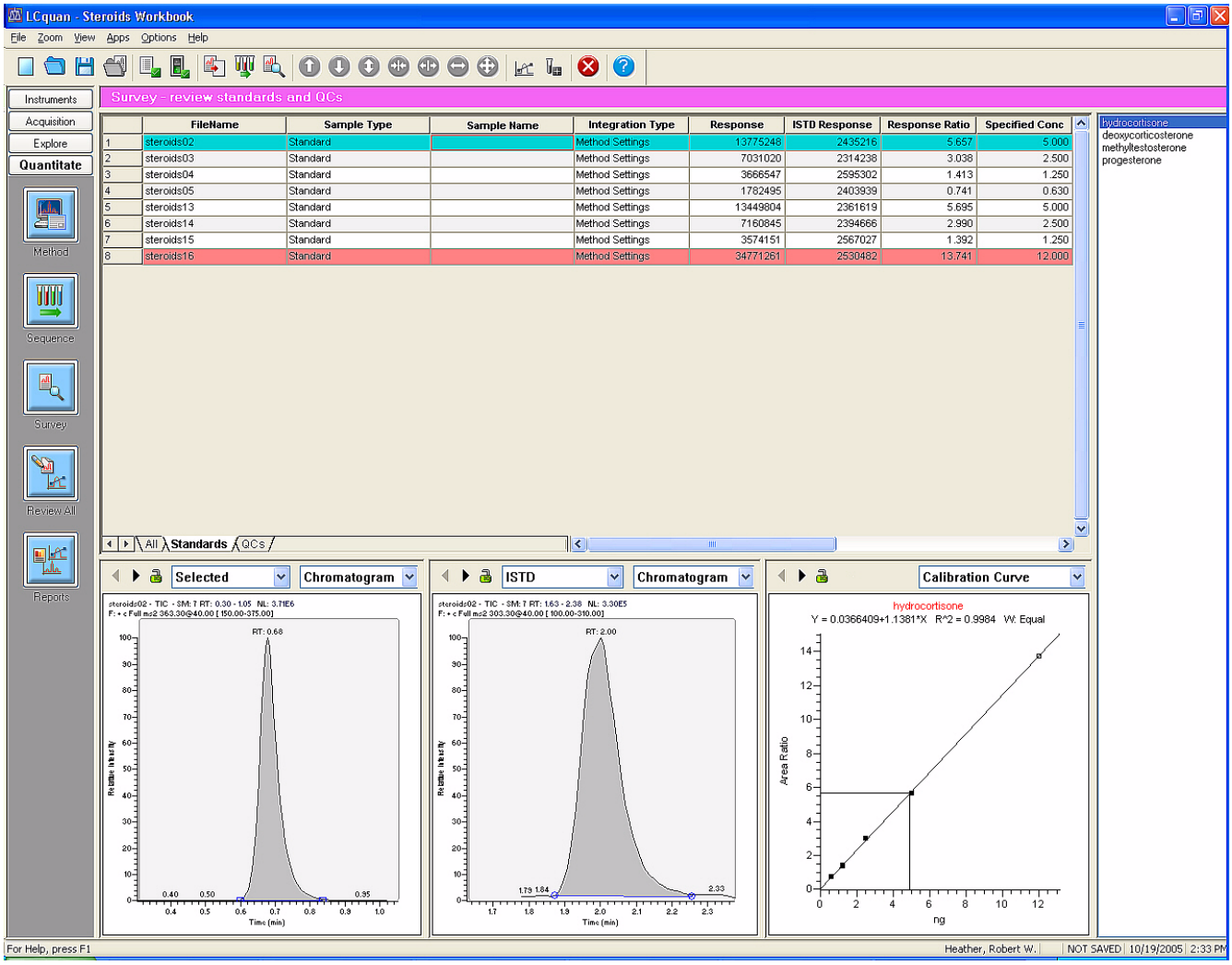
  Ensure that the target compound is selected in the Component List. Right-click in the Calibration Curve pane to display the shortcut menu. Then, choose **Exclusion List** from this menu to display the Calibration Exclusion List dialog box. For each standard that you want to exclude from the calibration curve, click in the Exclude column of its row. The word *Yes* appears in the Exclude column. Click on **Apply** to incorporate the change. (To include the standard again, click on the word *Yes* and then click on **Apply**.)

- By using the Calibration Curve pane:

  Left-click on the data point in the Calibration Curve pane that you want to exclude, and then right-click to display the shortcut menu. Choose **Exclude** from this menu to exclude the point. (To include the standard again, left-click on the data point. Then, right-click and choose **Include** from the shortcut menu.)

When you exclude a calibration standard in any of the above ways, LCquan highlights in dark pink its row in the Result List and displays the excluded data point as an empty box in the Calibration Curve pane. See Figure 51.

Figure 51. Survey page of the Quantitate view, showing that the last calibration standard in the Result List has been excluded from the calibration curve


LCquan - Steroids Workbook
File Zoom View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Method
Sequence
Survey
Review All
Reports
Survey - review standards and QCs
FileName Sample Type Sample Name Integration Type Response ISTD Response Response Ratio Specified Conc
1 steroids02 Standard Method Settings 13775248 2435216 5.657 5.000
2 steroids03 Standard Method Settings 7031020 2314238 3.038 2.500
3 steroids04 Standard Method Settings 3666547 2595302 1.413 1.250
4 steroids05 Standard Method Settings 1782495 2403939 0.741 0.630
5 steroids13 Standard Method Settings 13449804 2361619 5.695 5.000
6 steroids14 Standard Method Settings 7160845 2394666 2.990 2.500
7 steroids15 Standard Method Settings 3574151 2567027 1.392 1.250
8 steroids16 Standard Method Settings 34771261 2530482 13.741 12.000
All Standards QCs
hydrocortisone
deoxycorticosterone
methyltestosterone
progesterone
Selected Chromatogram
ISTD Chromatogram
Calibration Curve
RT: 0.68
RT: 2.00
hydrocortisone
Y = 0.0366409+1.1381*X R^2 = 0.9984 W: Equal
Area Ratio
ng
Time (min)
For Help, press F1
Heather, Robert W.
NOT SAVED 10/19/2005 2:33 PM

Modifying the Peak Detection and Integration Settings

You can modify the peak detection and integration settings from the Survey view (or the Review All view).

❖ To modify the peak detection and integration settings

1. Select a sample by clicking on the appropriate row in the Result List.
2. Right-click in the Chromatogram pane to display the shortcut menu.
3. From the shortcut menu, choose **User Peak Detection Settings** to open the User Identification Settings dialog box.
4. To modify the peak detection settings, click on the **Detection** tab and change the settings as necessary.
5. To modify the peak integration settings, click on the **Integration** tab. Then, change the settings as necessary to correct problems with noise in the peak, unresolved peaks, or peak tailing.
6. To modify the advanced peak detection parameters, click on the **Advanced** tab. Then, adjust the settings as necessary to improve peak detection and integration if baseline noise causes interference.

   See the online Help for further information about these advanced parameters.
7. Click on **Apply** to apply the changes to the sample that is currently selected in the Result List, or click on **Apply To All** to apply the changes to all of the samples on the current page of the Result List (for example, to all samples on the Standards page).
8. When you are finished, click on **OK** to close the dialog box.

   If you changed any parameters since you last clicked on the Apply button or the Apply To All button, clicking on OK will apply the new changes to the sample selected in the Result List.

When you modify the peak detection and integration parameters, LCquan displays *User Integration* in the Integration Type column of the Result List.

To revert back to the original peak detection and integration parameters from the Processing Method, do the following: In the Integration Type column of the Result List, click in the cell containing the words *User Integration* for the sample that you want to change. Then, click on the down arrow and select *Method Settings* from the drop-down list.

Integrating Peaks Manually

You can manually change the baseline and the starting and ending points of a peak in either of two ways:

- By clicking and dragging the starting and ending points:

  To move a starting or ending point of a peak, position the cursor over one of the integration markers. [The cursor turns into crosshairs (+).] Then, click and drag the integration marker to the desired location.

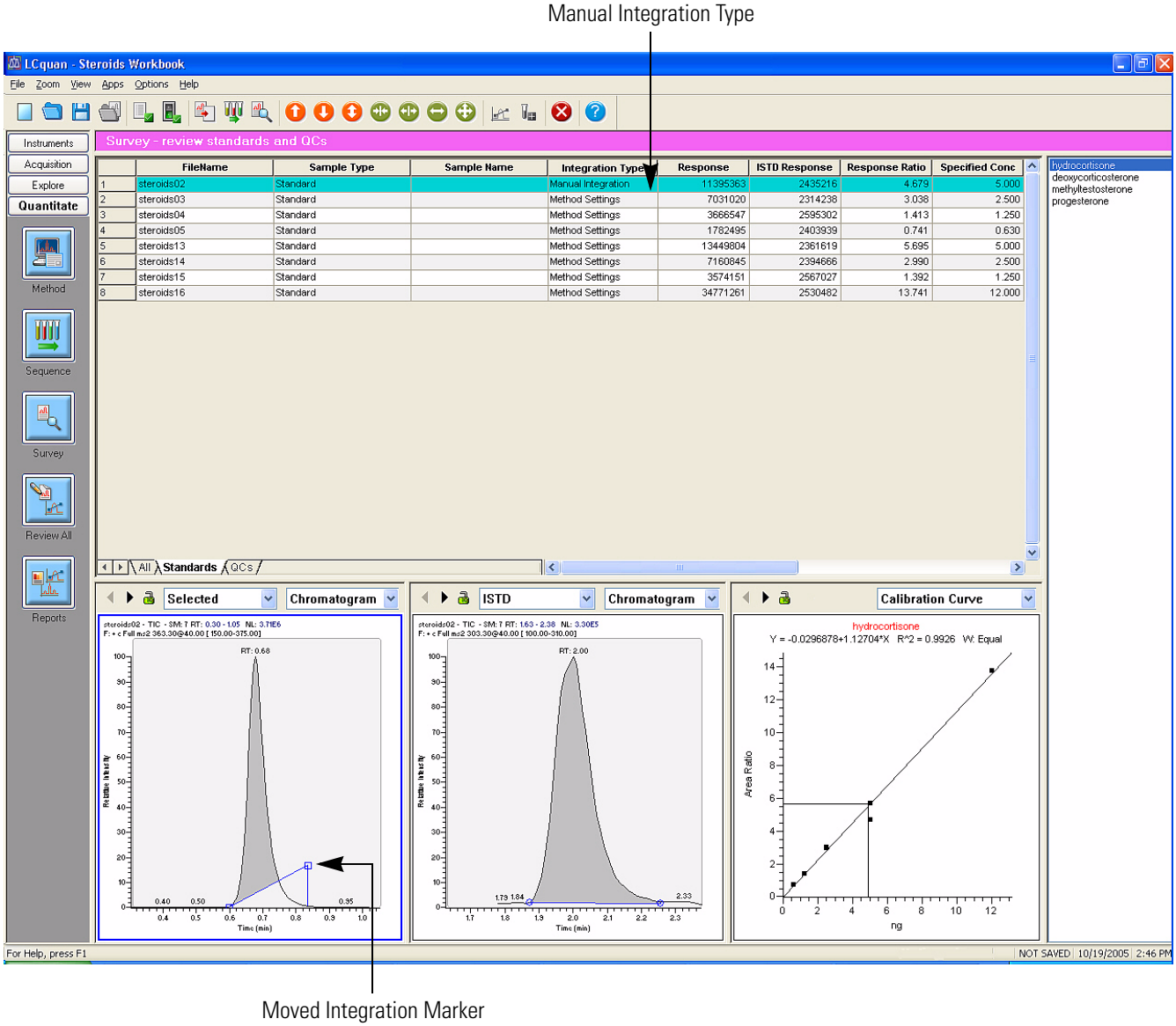
- By using the Baseline dialog box:

  To precisely position a starting or ending point of a peak, right-click in the Chromatogram pane to display the shortcut menu. Choose **Change Peak Baseline** to open the Baseline dialog box. Enter the new coordinates for the starting and ending points into the appropriate text boxes and click on **OK**.

When you integrate a peak manually, LCquan displays *Manual Integration* in the Integration Type column of the Result List. See Figure 52.

You can easily undo the manual integration by using the Integration Type column of the Result List: In the Integration Type column, click in the cell containing the words *Manual Integration* for the sample that you want to change. Then, click on the down arrow and select *Method Settings* from the drop-down list to return the integration to its prior settings.

Figure 52. Survey page of the Quantitate view, showing a manually integrated peak in the Chromatogram pane


Manual Integration Type
LCquan - Steroids Workbook
File Zoom View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Method
Sequence
Survey
Review All
Reports
Survey - review standards and QCs
FileName | Sample Type | Sample Name | Integration Type | Response | ISTD Response | Response Ratio | Specified Conc
1 steroids02 Standard Manual Integration 11395363 2435216 4.679 5.000
2 steroids03 Standard Method Settings 7031020 2314238 3.038 2.500
3 steroids04 Standard Method Settings 3666547 2595302 1.413 1.250
4 steroids05 Standard Method Settings 1782495 2403939 0.741 0.630
5 steroids13 Standard Method Settings 13449804 2361619 5.695 5.000
6 steroids14 Standard Method Settings 7160845 2394666 2.990 2.500
7 steroids15 Standard Method Settings 3574151 2567027 1.392 1.250
8 steroids16 Standard Method Settings 34771261 2530482 13.741 12.000
hydrocortisone
deoxycorticosterone
methyltestosterone
progesterone
All Standards QCs
Selected Chromatogram
ISTD Chromatogram
Calibration Curve
RT: 0.68
RT: 2.00
hydrocortisone
Y = -0.0296878+1.12704*X R^2 = 0.9926 W: Equal
Area Ratio
ng
Time (min)
For Help, press F1
NOT SAVED 10/19/2005 2:46 PM
Moved Integration Marker

Reviewing All Results

You use the Review All Results page of the Quantitate view to review and rework samples of all types, including unknowns and blanks. The process of reviewing the unknowns and blanks involves the following steps:

- Opening the Review All Results Page
- Reviewing the Unknowns

If necessary, you can rework the samples by modifying the peak detection and integration settings and by integrating peaks manually.

For calibration standards and QCs, the Review All Results page functions exactly as the Survey page does. (Refer to "Reviewing the Calibration Curve and QCs" on page 100.)

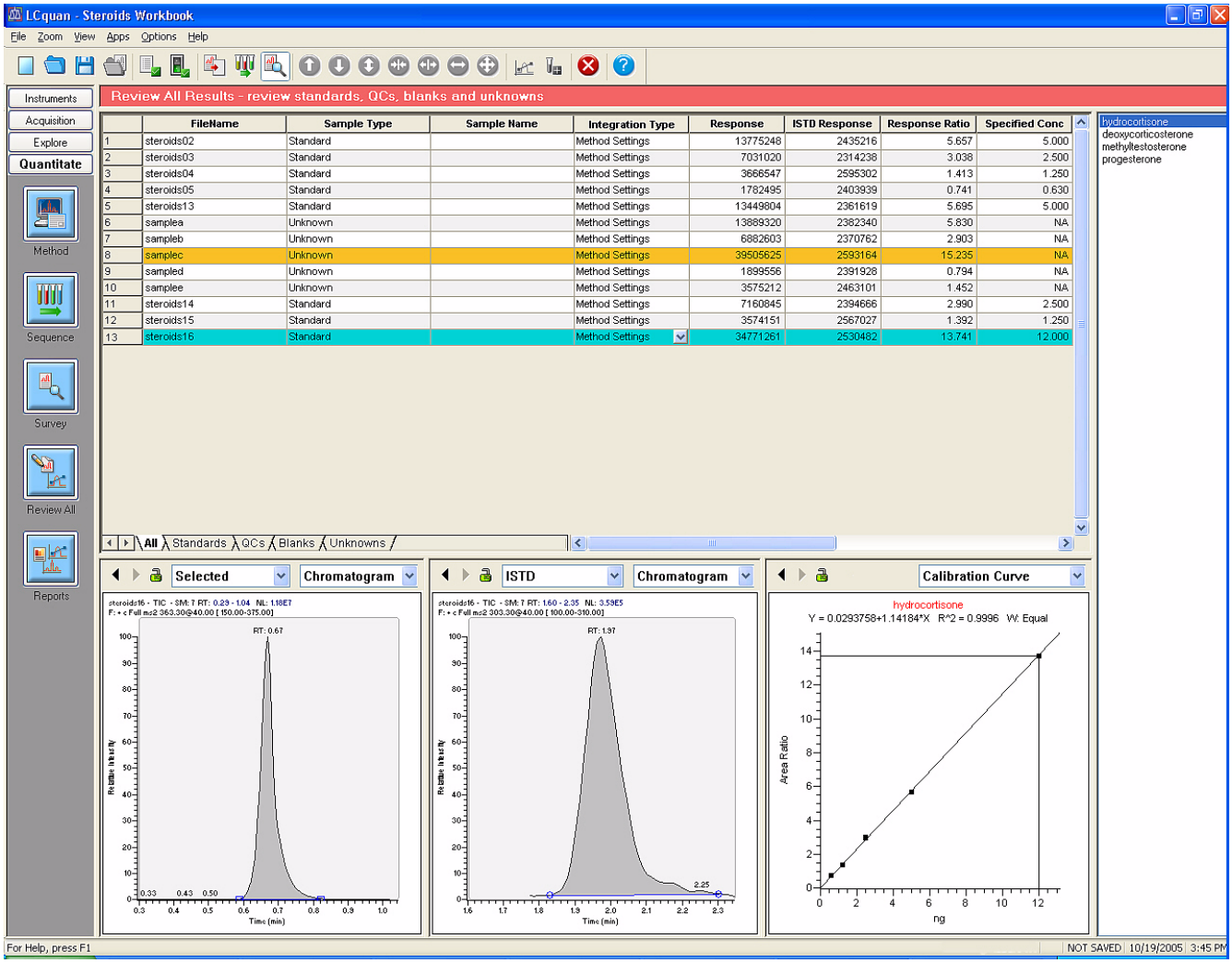
Note The steroids data set does not contain blank samples.

Opening the Review All Results Page

To process the raw files and open the Review All Results page, click on the **Review All** button in the viewbar. See Figure 53.

The Review All Results page includes (clockwise from the top) the Result List, the Component List, the Calibration Curve pane, the ISTD Chromatogram pane, and the Chromatogram pane. These elements function exactly as they do in the Survey page. The only difference is that the Result List now has five display tabs: All, Standards, QCs, Blanks, and Unknowns. Clicking on the All tab displays the results for every sample in the sequence.

Figure 53. Review All Results page of the Quantitate view, showing the results for the steroids data


LCquan - Steroids Workbook
File Zoom View Apps Options Help
Instruments
Acquisition
Explore
Quantitate
Method
Sequence
Survey
Review All
Reports
Review All Results - review standards, QCs, blanks and unknowns
FileName | Sample Type | Sample Name | Integration Type | Response | ISTD Response | Response Ratio | Specified Conc
1 steroids02 Standard Method Settings 13775248 2435216 5.657 5.000
2 steroids03 Standard Method Settings 7031020 2314238 3.038 2.500
3 steroids04 Standard Method Settings 3666547 2595302 1.413 1.250
4 steroids05 Standard Method Settings 1782495 2403939 0.741 0.630
5 steroids13 Standard Method Settings 13449804 2361619 5.695 5.000
6 samplea Unknown Method Settings 13889320 2382340 5.830 NA
7 sampleb Unknown Method Settings 6882603 2370762 2.903 NA
8 samplec Unknown Method Settings 39505625 2593164 15.235 NA
9 sampled Unknown Method Settings 1899556 2391928 0.794 NA
10 samplee Unknown Method Settings 3575212 2463101 1.452 NA
11 steroids14 Standard Method Settings 7160845 2394666 2.990 2.500
12 steroids15 Standard Method Settings 3574151 2567027 1.392 1.250
13 steroids16 Standard Method Settings 34771261 2530482 13.741 12.000
hydrocortisone
deoxycorticosterone
methyltestosterone
progesterone
All Standards QCs Blanks Unknowns
Selected Chromatogram
RT: 0.67
Time (min)
ISTD Chromatogram
RT: 1.97
Time (min)
Calibration Curve
hydrocortisone
Y = 0.0293758+1.14184*X R^2 = 0.9996 W: Equal
Area Ratio
ng
For Help, press F1
NOT SAVED 10/19/2005 3:45 PM

Reviewing the Unknowns

❖ To review and rework the results for the unknowns

1. Click on the **Unknowns** tab at the bottom of the Result List to display the peak integration results for the unknown samples.
2. In the Component List, select the internal standard, ***methyltestosterone***.
3. Inspect each file in the Result List and check for peak detection and integration problems.
4. Inspect the chromatogram in the Chromatogram pane for each file.
   a. Ensure that LCquan found the peak.
   b. Ensure that the shaded area accurately represents the contribution of the component to the chromatogram.
      - If you want to modify the peak detection and integration settings, go to the topic: "Modifying the Peak Detection and Integration Settings" on page 109.
      - If you want to integrate a peak manually, go to the topic: "Integrating Peaks Manually" on page 110.
5. In the Component List, select a target compound.
6. Repeat steps step 3 and step 4 above for each target compound.

   Note For the file *samplec*, target compound *hydrocortisone*, its row in the Result List is shaded pale orange and the Peak Status column displays ***Response High***. This means that the response for the sample is greater than the response for any of the calibration standards.

7. When you are satisfied that LCquan has detected and integrated the peaks properly, inspect the Result List again. The calculated amount of steroids in each unknown sample is displayed in the Calculated Amount column of the list. See Figure 54.
8. Left-click in the Chromatogram pane to select it. Then, right-click and choose **Show Peak Info** from the shortcut menu. The Peak Information dialog box opens and allows you to review the peak properties for the selected steroids sample. See Figure 55.

   With the Peak Information dialog box still open, select the other steroids files in the Result List and view the peak properties for them. For more information about the peak properties, refer to the online Help.

Figure 54. Review All Results page of the Quantitate view, showing the results for the hydrocortisone unknowns

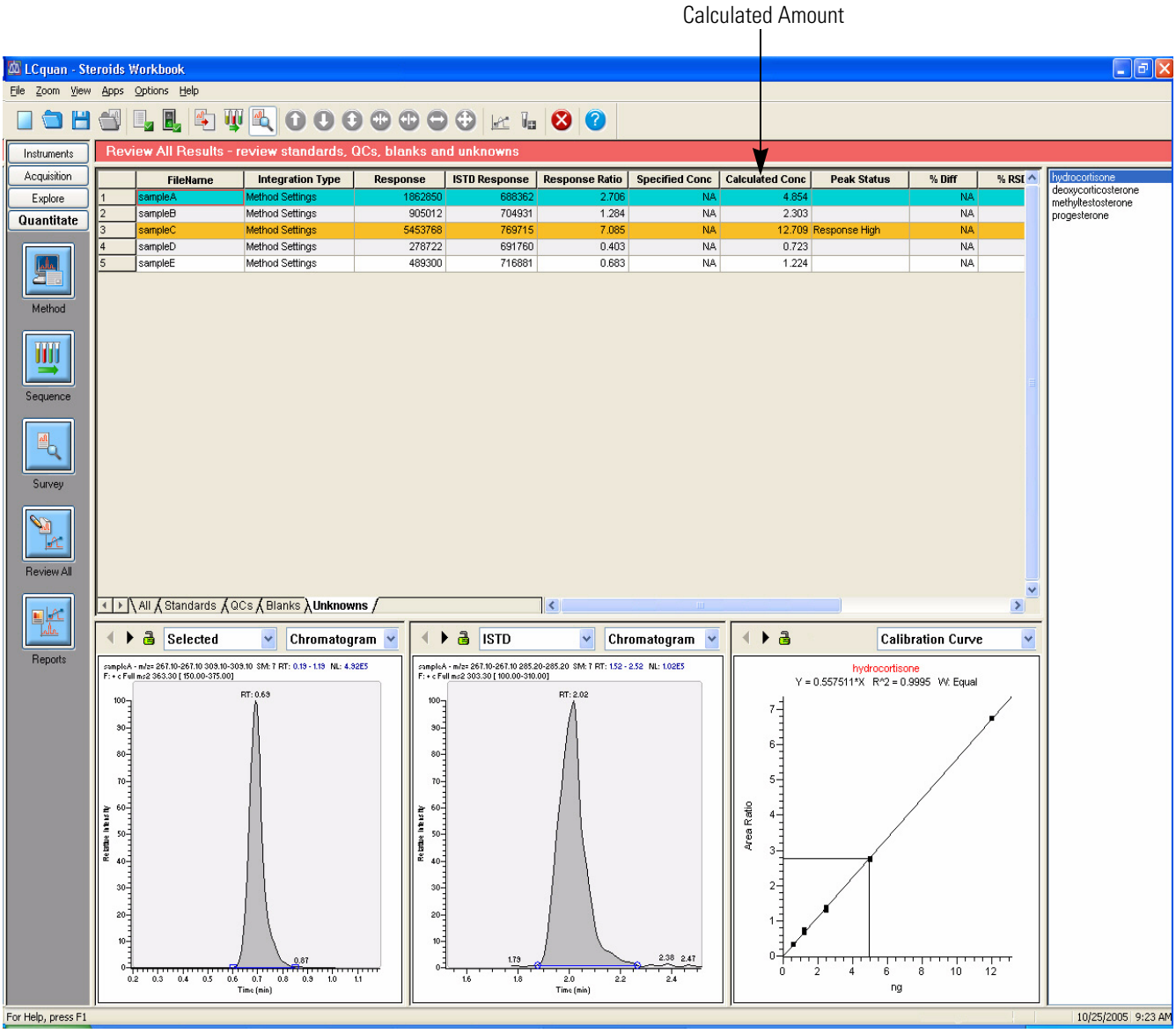
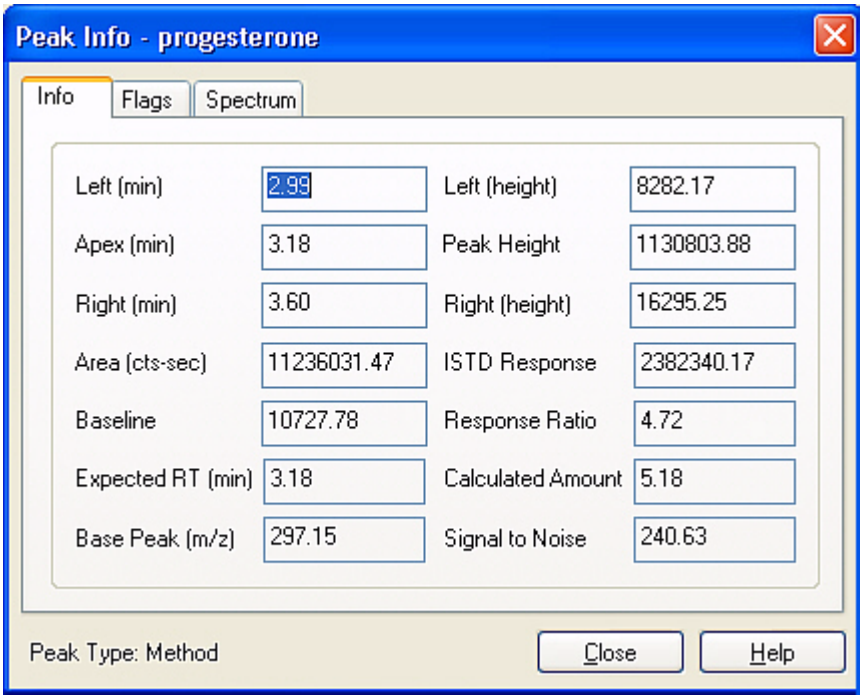


Figure 55. Peak Information dialog box, showing the progesterone peak properties for the samplea raw file



Creating and Reviewing Reports

You use the Review Reports page of the Quantitate view to create and review reports describing the quantitative analysis results for your data.

❖ To create and review reports for your steroids data

1. Click the **Reports** button in the viewbar. The Review Reports page appears. See Figure 56.
2. To specify Microsoft Excel® reports, do the following:
   a. In the **Excel Report Selections** table, select the **Use** check box in an empty row.
   b. In the **Column Arrangement** column, select a column arrangement from the list.

      Three column arrangements are available by default: Quan Result Grid, Excel Long Summary, and Excel Short Summary. See Table 8 on page 119 for a description of each report type.
   c. To create additional Excel Reports, repeat steps a and b.
   d. To create Sample Style Excel Reports (reports with information on one sample per page, rather than the default one component per page), select the **Sample Style Excel Reports** check box.

3. To specify reports using the XReport templates, do the following:
   a. In the **XReport Report Selections** table, select the **Use** check box in an empty row.
   b. In the **Save Type** column, select the file format in which you want to save the report.
   c. In the **XReport Template** column, type the name of an XReport template, or click the empty cell, and then click to browse for a template. The standard report templates contain preselected methods and results summaries. See Table 8 on page 119.

      Alternatively, click Create XReport Templates to create a custom report template.

      Tip To preview reports with your data, open a template, and then choose **Report > Simulate Report**.

      For information about XReport, refer to *Getting Productive: Designing and Generating Custom Reports with XReport*.

   d. To create additional reports with XReport, repeat steps a through c.

      Note If your administrator has set up secure reporting, the Create XReport Templates button is unavailable, and only the templates in the secure template folder are available. The only output option is Save Only, which saves the file in pdf format.

4. In the Select Report Options area, select the output option. You can choose Print Only, Save Only, or Print and Save.
5. Click on Create Reports to generate the specified reports.

   Note When you save a report using the Save Only or the Print and Save option, LCquan places a copy of the report in the Exports folder of the workbook.

Figure 56. Review Reports page of the Quantitate view, showing the report options

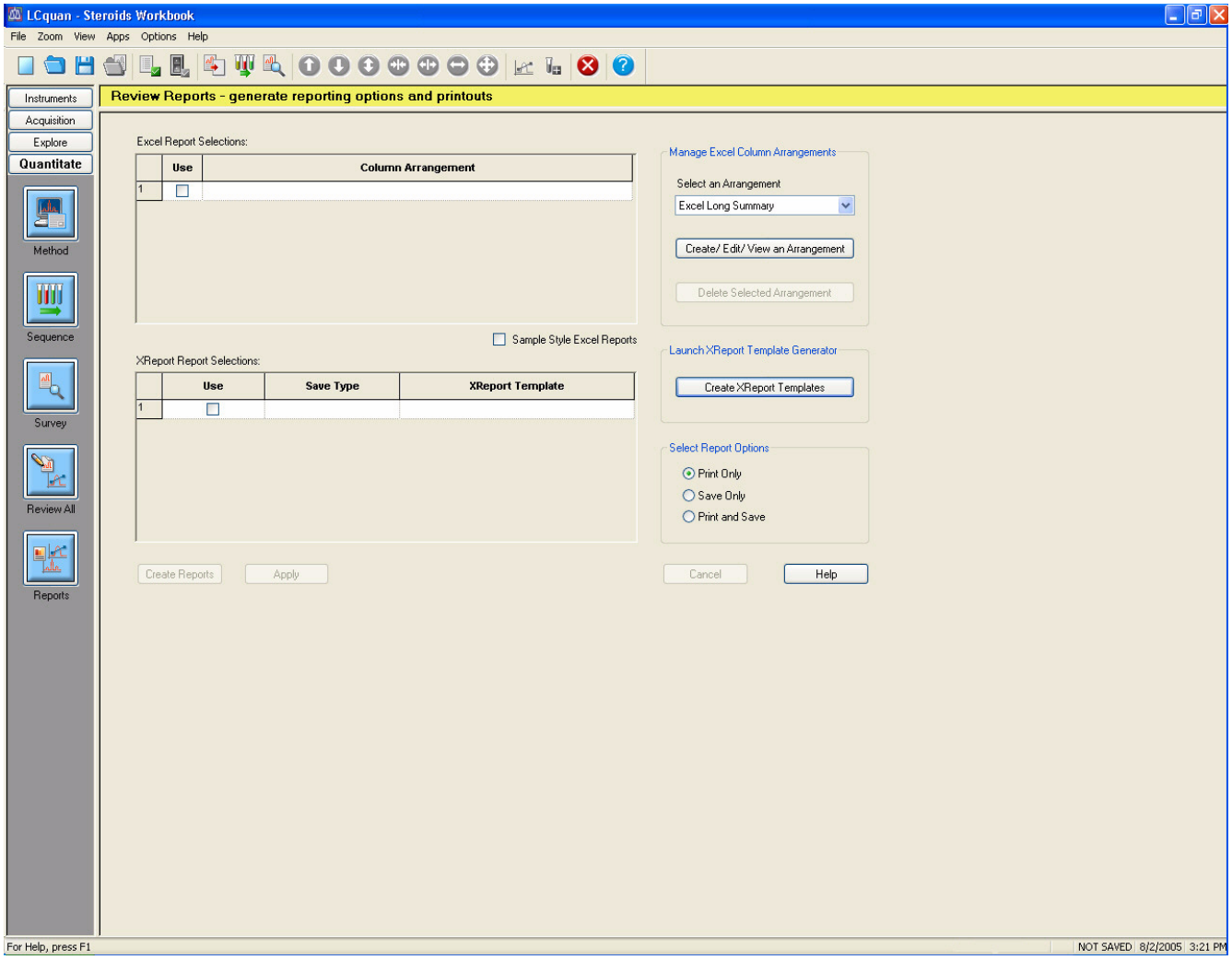


Table 8. Report options available in Review Reports page (Sheet 1 of 2)

Report Options	Description
XReport Selections*	
Acquisition Sequence Report (LCquanAcqSequence.xrt)	Displays the sample information that is in the Acquisition Sequence, for example, sample ID, raw file names, sample types, and cal levels.
Component Calibration Report (Avalon, Genesis, or ICIS algorithm) (LCquanCompCalReport.xrt)	Provides the component identification and calibration parameters, calibration curves, calibration and QC levels and amounts, and Result List information (for example, peak areas and calculated amounts) for all components in all samples.
Ion Ratio Conformation Results Report (LCquanIonRatioConformationGraphical.xrt)	Provides the chromatogram traces of the conformation ion and qualifier ions for each component. The report also lists sample information, peak status, and calculated concentrations for each sample.
Peak Integration Report (LCquanPeakIntegration.xrt)	Provides the Results List information (for example, peak areas and calculated amounts) for all components, the sample information, and the Instrument Method name and Processing Method name, if applicable. The report includes plots of the chromatogram peaks for all components.
Processing Method Report (Avalon, Genesis, or ICIS algorithm) (LCquanProcessingMethod.xrt)	Lists all the peak detection and integration parameters in the Processing Method for each component. The report also lists level amounts, IRC data, and the mass-to-charge ratios and intensities of the peaks in the mass spectrum for each component.
Quan Components Peak Results Report (LCquanQuanPeakResults_ESTD.xrt, LCquanQuanPeakResults_ISTD.xrt)	Lists the sample information, acquisition conditions, and quantitative analysis results for a single sample. The report also displays the integrated chromatograms of each target compound and the standard, as well as the calibration curves.
Quantify Component Report (LCquanQuantifyComponentReport.xrt)	Provides a summary by component of the samples for each component. The report includes sample information and quantitative analysis results (for example, peak areas, response, and calculated amount), and it displays the integrated chromatogram peaks.
Quantify Sample Report (LCquanQuantifySampleReport.xrt)	Provides a summary by sample of the components for each sample. The report includes sample information and quantitative analysis results (for example, peak areas, response, and calculated amount), and it displays the integrated chromatogram peak.
Quan Results Report (LCquanQuanResults.xrt)	Provides quantitative results for a single sample.

Table 8. Report options available in Review Reports page (Sheet 2 of 2)

Report Options	Description
Excel Report Options	
Quan Result Grid	Generates an Excel report using the current Quan Results column arrangement as specified on the Survey and Review pages of the Quantitate section. This column arrangement cannot be modified from the Review Reports page.
Short Excel Summary	Displays the Result List of the Quantitate view in a Microsoft Excel spreadsheet. Each component is placed on a separate page/worksheet in the main workbook. You can review, analyze, and graph the data by using all of the functions and features of Microsoft Excel.
Long Excel Summary	Displays the Result List of the Quantitate view, Processing Sequence, and component identification and calibration information in a Microsoft Excel spreadsheet. Each component is placed on a separate page/worksheet in the main workbook. You can review, analyze, and graph the data by using all of the functions and features of Microsoft Excel.

*If your administrator has set up secure reporting, the Create XReport Templates button is unavailable, and only the templates in the secure template folder are available. The only output option is Save Only, which saves the file in pdf format.

Locking the Workbook

You can create a locked version of a workbook at any time, except when data is being acquired. A ***locked workbook*** is essentially a copy of the workbook (and its associated files) that cannot be overwritten. You cannot save any changes made to a locked workbook, and you cannot acquire data in a locked workbook. You can create new reports, but LCquan does not save the report selections.

❖ To lock the current workbook

Choose **File > Create Locked Version of Workbook**. LCquan creates a copy of the workbook and its associated files under the current study folder.

The locked workbook is renamed by appending "_locked_*nnn*" to the original workbook name, where *nnn* is the number of the locked workbook. For example, the first time that you create a locked workbook from the workbook *Trial1*, LCquan names the locked workbook *Trial1_locked_001*. The second time that you lock the workbook *Trial1*, LCquan names the locked workbook *Trial1_locked_002.* You can create up to 999 locked versions of a workbook. You cannot unlock a locked workbook.

When you open a locked workbook, LCquan displays *[Locked]* in the title bar next to the workbook name and in the status bar.

Saving the Workbook and Exiting LCquan

You can save the workbook at any step in the LCquan procedure. This allows you to exit LCquan and reenter at the same place at a later time. When you reopen a workbook, LCquan displays the page and view that were open when you exited the workbook.

❖ To save the workbook and exit LCquan

1. Choose **File > Save** to save the current workbook.

   If you want to save the workbook with a different study or workbook name, choose **File > Save As**. The Save As Wizard appears to guide you through the procedure.

2. Choose **File > Exit** to exit LCquan.

Note If you acquire data, LCquan will not let you exit the program unless you save the workbook.

LCquan Flow Diagrams

This appendix contains a set of flow diagrams for LCquan for you to use as a reference. The flow diagrams show step-by-step progressions through the different views of LCquan.

The following flow diagrams are contained in this appendix:

- LCquan flow diagram
- LCquan for Citrix flow diagram
- Acquisition view flow diagram
- Quantitate view flow diagram

Figure 57. LCquan flow diagram, showing the steps for creating a new study or workbook and the four main views in dark gray

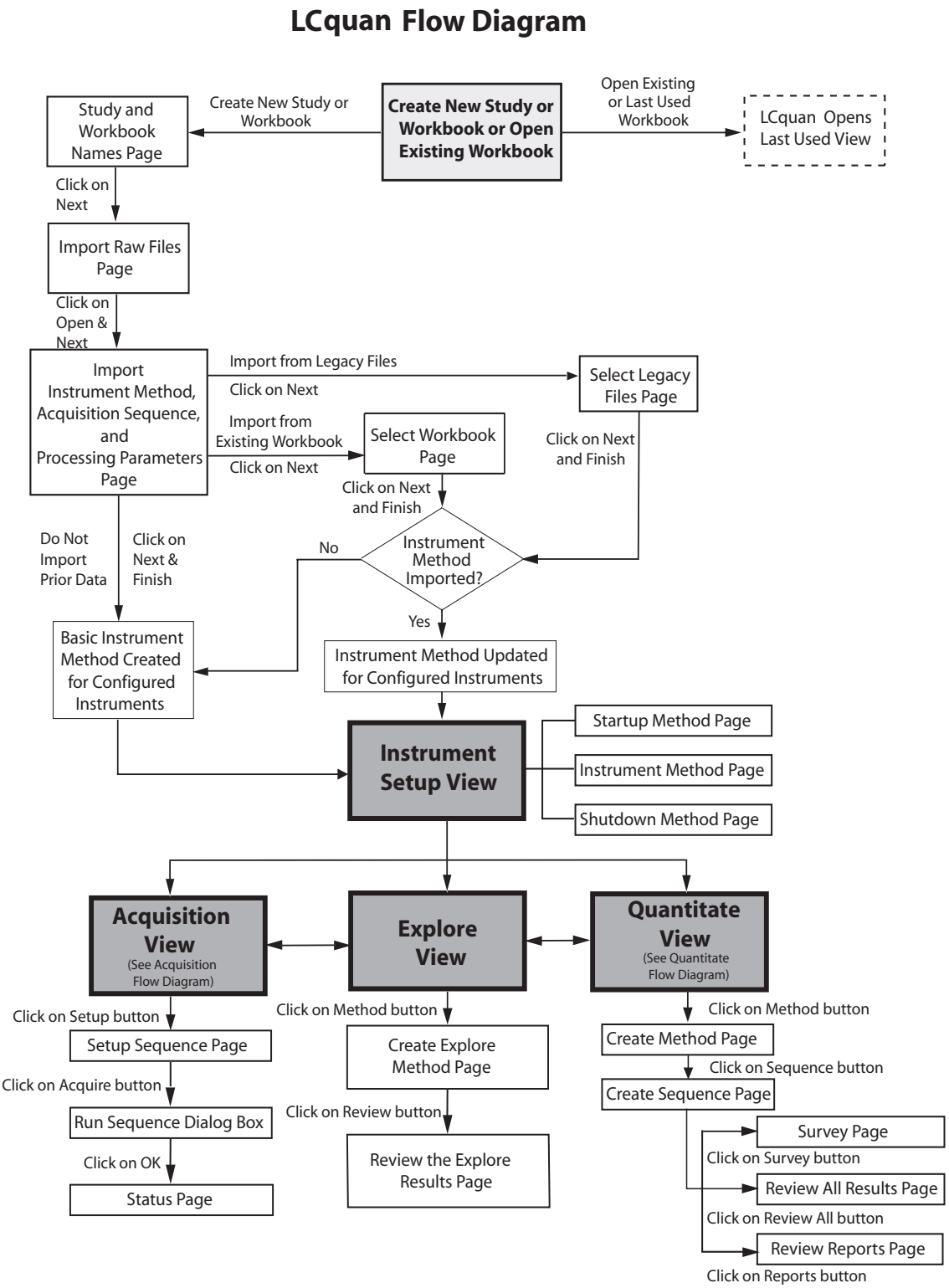


Figure 58. LCquan for Citrix flow diagram, showing the steps for creating a new study or workbook and the two main views in dark gray

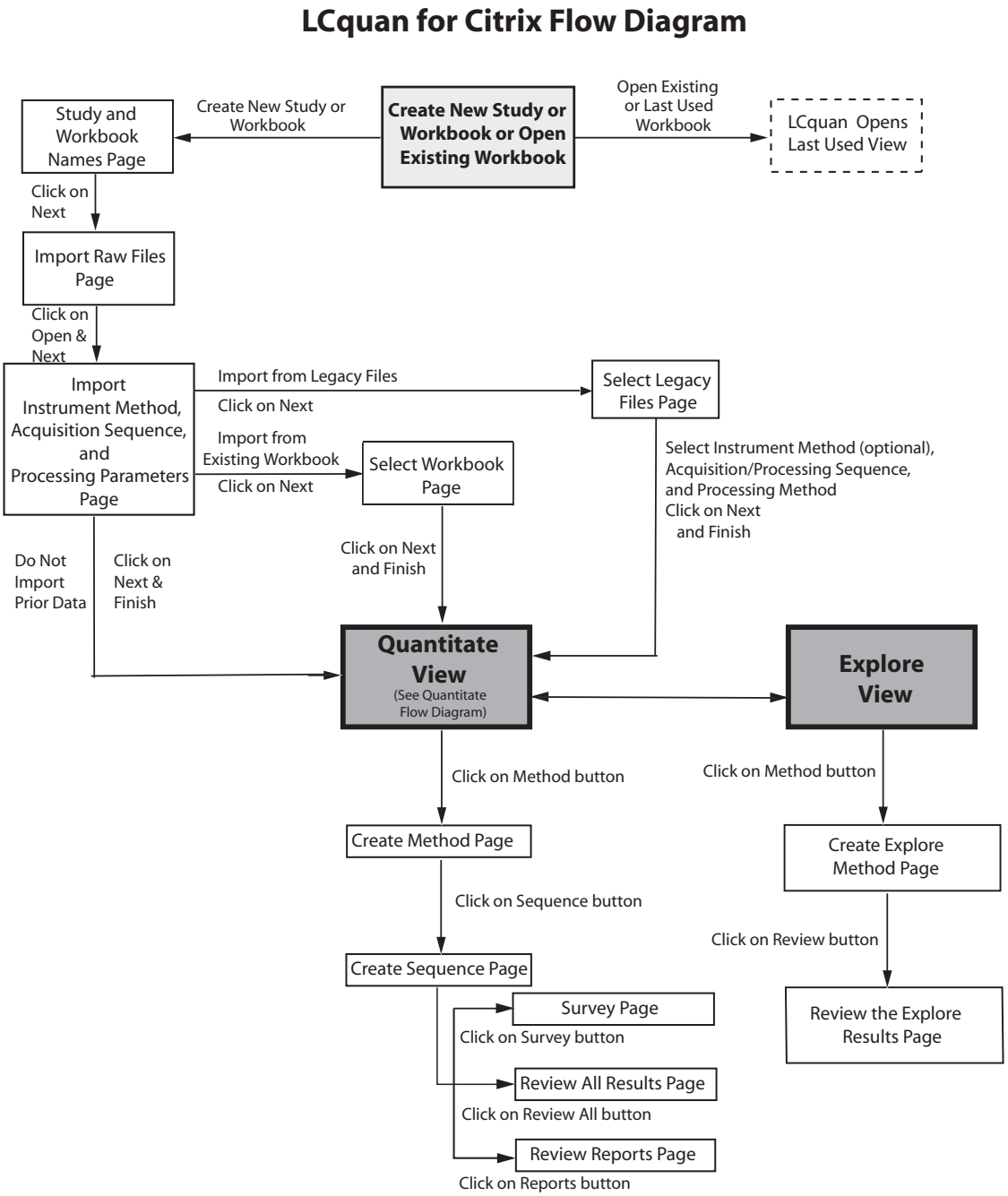

LCquan for Citrix Flow Diagram
Create New Study or Workbook or Open Existing Workbook
Create New Study or Workbook
Study and Workbook Names Page
Open Existing or Last Used Workbook
LCquan Opens Last Used View
Click on Next
Import Raw Files Page
Click on Open & Next
Import Instrument Method, Acquisition Sequence, and Processing Parameters Page
Import from Legacy Files
Click on Next
Select Legacy Files Page
Import from Existing Workbook
Click on Next
Select Workbook Page
Select Instrument Method (optional), Acquisition/Processing Sequence, and Processing Method
Click on Next and Finish
Do Not Import Prior Data
Click on Next & Finish
Click on Next and Finish
Quantitate View
(See Quantitate Flow Diagram)
Explore View
Click on Method button
Click on Method button
Create Method Page
Create Explore Method Page
Click on Sequence button
Click on Review button
Create Sequence Page
Survey Page
Click on Survey button
Review the Explore Results Page
Review All Results Page
Click on Review All button
Review Reports Page
Click on Reports button

Figure 59. Acquisition view flow diagram

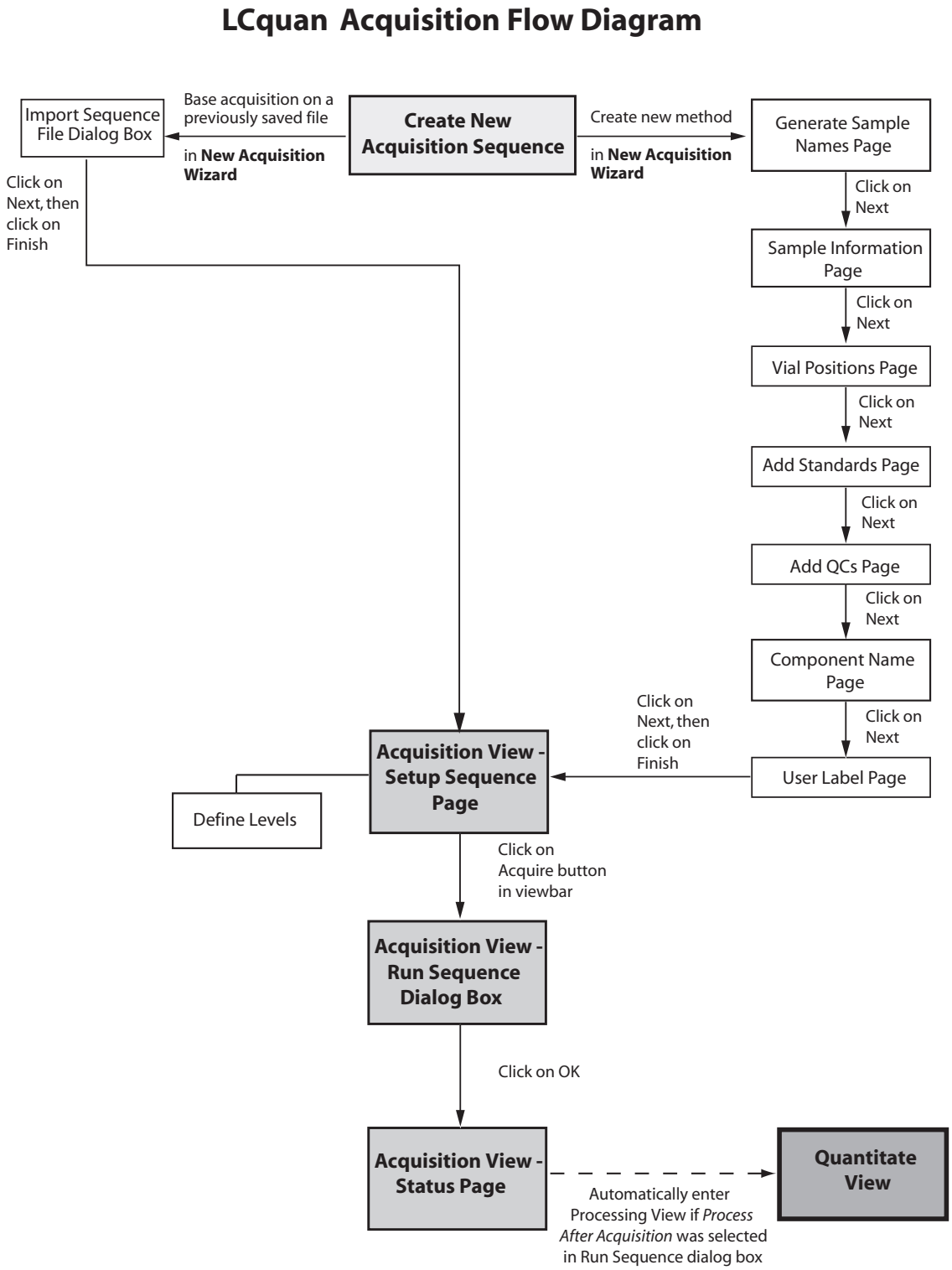
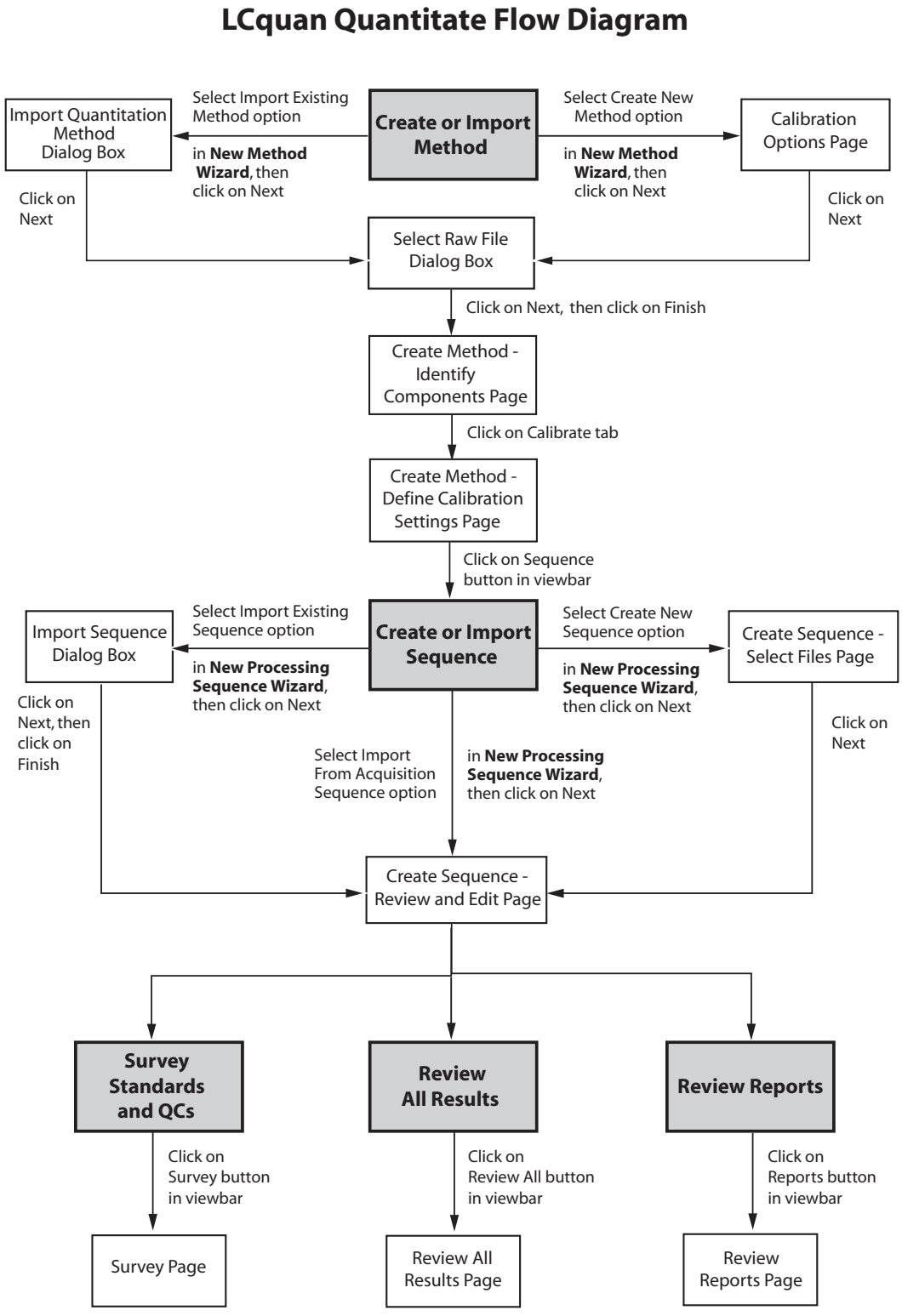

LCquan Acquisition Flow Diagram
Create New Acquisition Sequence
Base acquisition on a previously saved file
in New Acquisition Wizard
Import Sequence File Dialog Box
Click on Next, then click on Finish
Create new method
in New Acquisition Wizard
Generate Sample Names Page
Click on Next
Sample Information Page
Click on Next
Vial Positions Page
Click on Next
Add Standards Page
Click on Next
Add QCs Page
Click on Next
Component Name Page
Click on Next
User Label Page
Click on Next, then click on Finish
Acquisition View - Setup Sequence Page
Define Levels
Click on Acquire button in viewbar
Acquisition View - Run Sequence Dialog Box
Click on OK
Acquisition View - Status Page
Automatically enter Processing View if Process After Acquisition was selected in Run Sequence dialog box
Quantitate View

Figure 60. Quantitate view flow diagram


LCquan Quantitate Flow Diagram
Import Quantitation Method Dialog Box
Select Import Existing Method option
in New Method Wizard, then click on Next
Create or Import Method
Select Create New Method option
in New Method Wizard, then click on Next
Calibration Options Page
Click on Next
Click on Next
Select Raw File Dialog Box
Click on Next, then click on Finish
Create Method - Identify Components Page
Click on Calibrate tab
Create Method - Define Calibration Settings Page
Click on Sequence button in viewbar
Import Sequence Dialog Box
Select Import Existing Sequence option
in New Processing Sequence Wizard, then click on Next
Create or Import Sequence
Select Create New Sequence option
in New Processing Sequence Wizard, then click on Next
Create Sequence - Select Files Page
Click on Next, then click on Finish
Select Import From Acquisition Sequence option
in New Processing Sequence Wizard, then click on Next
Click on Next
Create Sequence - Review and Edit Page
Survey Standards and QCs
Review All Results
Review Reports
Click on Survey button in viewbar
Click on Review All button in viewbar
Click on Reports button in viewbar
Survey Page
Review All Results Page
Review Reports Page

Index

A

acquiring data
  overview 17
  procedure 44
  saving workbook after (note) 45
Acquisition Levels dialog box
  illustrated 36, 37, 41
  using 41
Acquisition Queue 47
Acquisition Sequence
  copying Processing Sequence to 38
  copying to Processing Sequence 96
  creating 30
  definition 28
  editing 39
  exporting 42
  illustrated 35
  importing 38
  printing 42
Acquisition Sequence History 48, 63
Acquisition view
  opening 30
  overview 27
Acquisition view – Setup Sequence page
  See Setup Sequence page.
Acquisition view – Status page
  See Status page.
analyte detection using mass spectrometry 4
associating raw sample files with sample types 89
audit trail, Explore view 52
Avalon peak detection 77

B

blanks 10

C

calibration curve
  curve types 15
  described 5
  excluding standards 107
  inspecting at low concentrations 102
  steroids (figure) 106
  using external standard 7
  using internal standard (illustrated) 9
  weighting 15
Calibration Curve pane 102
calibration curve, selecting points 103
calibration curve, showing only low concentration points 103
calibration levels
  assigning 36, 41
  associated steroids raw files and (table) 91
  matching names (note) 32, 32, 38
calibration levels and amounts for steroids 34
calibration standards 9
Chromatogram pane
  overview
    Acquisition Status view 48
    in Survey page 102
  using
    Review All Results page 114
    Status page 48
Chromatogram pane and Spectrum pane
  Acquisition view 50
Citrix, LCquan for 19
colors in Result List 102
comparing plots 65
Component List 102
components
  creating from peak list 61
  exporting names from peak list 56
  naming in Processing Method 75
configuring instruments 26
control buttons and functions, Acquisition view 47
copying
  Acquisition Sequence to Processing Sequence 96
  Processing Sequence to Acquisition Sequence 38
Create Method – Define Calibration Settings page
  illustrated 76
Create Method – Identify Components page 80

Create Sequence – Review and Edit page
  using 97
Create Sequence – Select Files page
  illustrated 93
  opening 88
  using 89

D

deoxycorticosterone
  calibration curve (figure) 106
  creating quan component 61
  naming 76, 77
  peak list (illustrated) 61
detection limit 6
documentation vii

E

editing
  Acquisition Sequence 39
  peak detection and integration settings 109
  Processing Sequence 97
Excel reports 116
Explore Sequence 63
Explore view
  additional information 62
  audit trail 52
  general parameter plots 66
  introduction 52
  locks 65
  review displays 69
  sample selector 63
  status plots 67
  status reports 68
  steroids example 52
exporting, Acquisition Sequence 42
external standards defined 7

F

file and folder structure 16
File Information pane (note) 91
flow diagrams
  acquiring data 126
  creating study or workbook 124
  creating study or workbook (Citrix) 125
  processing data 127

G

general parameters plots, Explore view 66
Genesis peak detection 77

H

hydrocortisone calibration curve (figure) 106

I

ICIS peak detection 77
Identify Components page 74
importing
  Acquisition Sequence 38
  Instrument Methods 27
  Processing Method 85
  Processing Sequence 95
  raw files 24
injection volume, autosampler 39
Instrument Methods
  creating 26
  defined 25
  importing 27
Instrument Setup view
  illustrated 25
  opening 24
  overview 25
Instrument Status 47
integrating peaks manually 110
integration markers 79
internal standards (ISTDs)
  defined 8
  using for quantitation 8
ion ratio confirmation (IRC)
  description 83
  hydrocortisone example 83
ion ratio confirmation (IRC) chromatogram 83
ISTD Chromatogram pane 102

L

liquid chromatography (LC)
  using for analyte separation 3
  variables for 3
locking plots for comparison 65
locking the workbook 121
locks against raw file change 65
lower quantitation limit 6

M

manual peak integration 111
manuals vii
mass spectrometry, analyte detection using 4
methyltestosterone (internal standard)
  calibration parameters 75
  naming 75

peak detection and integration parameters 78
modifiers, volatile 4

N

New Acquisition Wizard 30
New Method Wizard 72
New Processing Sequence Wizard 88
New Study or Workbook Wizard 23

O

overview of LCquan 10–18

P

peak detection type
Avalon 82
Genesis 82
ICIS 82
Peak Information dialog box, illustrated 116
peak lists
auto-generated names 56
creating quan components 61
deoxycorticosterone (illustrated) 61
exporting component names 56
generating 54
illustrated 55
renaming peaks 61
peaks
integrated (illustrated) 6
integrating manually 110
modifying integration parameters 109
properties, viewing 114
undoing manual integration of 110
printing, Acquisition Sequence 42
probes, API, selecting 4
processing
overview 17
raw sample files 99–110
while acquiring data 50
Processing Method
creating 71
defined 71
importing 85
saving 85
Processing Sequence
copying Acquisition Sequence to 96
copying to Acquisition Sequence 38
correcting errors in (note) 94
creating 87
defined 87
editing 97
importing 95
reviewing 94
progesterone calibration curve (figure) 106

Q

QC Level Names Association dialog box 96
QC levels
assigning 36, 41
matching names (note) 32, 32, 38
Qual Browser, starting from Explore view 70
quality control (QC) samples 10
quan components, creating from peak list 56, 61
Quantitate Sequence 63
quantitation limits 6
quantitation range 6
quantitative analysis
described 2–10
LCquan overview 17
sources of error 8
steps of 2
techniques 5
using external standards 7
using internal standards 8

R

raw sample files
associating with sample types 89
displaying in Chromatogram pane 48
importing into new workbook 24
locked and unlocked 65
processing 99–110
Realtime Display Settings dialog box 49
reports
creating 116–118
Microsoft Excel 116
options 119
XReport templates 116
response factor 5
Response High (note) 114
Result List
colors in (table) 102
description 100
Review All Results page
illustrated 113
opening 112
using 112
Review and Explore Results page 59
review displays, Explore view 69
review mode 22
Review Reports page 116
illustrated 118

opening 116
reviewing all results 112
reviewing calibration standards and QCs 102
Run Manager Status 47
Run Sequence dialog box
illustrated 43
opening 43

S

sample selector, Explore view 63, 64
sample types
blanks 10
quality controls (QCs) 10
standards 9
unknowns 9
saving workbook 121
scan filters, matching with components 77
Select Rawfile dialog box 73
semi-quantitative analysis, definition 2
sequence
See Acquisition Sequence, Processing Sequence.
Setup Sequence page
illustrated 29, 33
overview 28
Shutdown Methods
creating 26
defined 25
Spectrum pane
overview 48
Status page 49
Standard Level Names Association dialog box 96
standards
described 5
external standards 7
internal standards 8
sample types 9
starting LCquan 22
Startup dialog box 22
Startup Methods
creating 26
defined 25
Status page
illustrated 46
opening 45
overview 45
status plots, Explore view 67
status reports, Explore view 68
steroids example
01) creating the study and workbook 22
02) importing raw files 24
03) creating the Acquisition Sequence 30
04) editing the Aquisition Sequence 34
05) exploring the data 52
06) creating the Processing Method 71
07) create the Processing Sequence 88
08) processing the raw files 100
09) reviewing the calibration standards 102
10) reviewing the unknowns 114
Acquisition Sequence, edited 35
Acquisition Sequence, initial 29
calibration levels (table) 34
calibration levels and associated raw files (table) 91
calibration levels, assigning 36, 37, 41
file names (table) 34
introduction 18
most intense ions 60
peak detection and integration parameters 77
peak list 54
sample types (table) 34
study
creating 22
description 16
Survey page
illustrated 101, 108
manual integration 111
opening 100
using 100

T

templates, Explore view 69
templates, importing or exporting, Explore view 69
toolbar buttons for zooming (note) 104

U

unknowns 9
upper quantitation limit 6
user guides vii
user labels, changing 40

V

validation, Citrix 19
variables
quantitation with internal standards 8
separation by LC 3

W

workbook
creating 22, 30
description 16
locking 121
saving 121

X

XReport 116

Z

zooming
in Chromatogram pane 48
in Spectrum pane 50
toolbar buttons (note) 104